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(71) Applicant: **E.I. DU PONT DE NEMOURS AND COMPANY**
1007 Market Street
Wilmington Delaware 19898(US)

(72) Inventor: **Lung, Kaki Rick**
48 Quail Hollow Drive
Hockessin, Delaware 19707(US)
Inventor: **Fok, John Shiu-Kang**
41 Slashpine Circle
Hockessin, Delaware 19707(US)
Inventor: **Chanin, Alex Joseph**
8837 Rising Sun Avenue
Philadelphia, Pennsylvania 19115(US)

(74) Representative: **von Kreisler, Alek, Dipl.-Chem. et al**
Patentanwälte Von Kreisler-Selting-Werner, Deichmannhaus am Hauptbahnhof
W-5000 Köln 1(DE)

(54) Method for verifying the accuracy of a liquid dispenser.

(57) This invention relates to a method of of determining the accuracy of a liquid dispenser. More particularly, this invention relates to a method of use of robotic equipment in the processing of microliter-plate based assays. The method permits the diagnostic instruments using absorbance type measurements and laboratory robots in the analysis of samples to verify the accuracy of the dispensing portion of the robot. This method finds particular use in the diagnostic instruments analyzing samples which contain viruses (or antibody to the virus) of infectious diseases. Typical of such infectious diseases are the human immunodeficiency virus Type -1(HIV-1).

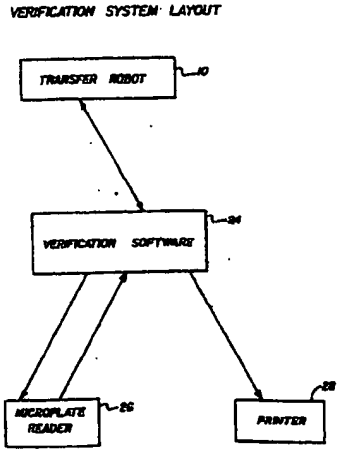


Fig. 3

EP 0 431 578 A2

LIQUID DISPENSER ACCURACY VERIFICATION METHOD

FIELD OF THE INVENTION

This invention relates to a method of determining the accuracy of a liquid dispenser. More particularly, this invention relates to a method of use of robotic equipment in the processing of microtiter-plate based assays. The method permits diagnostic instruments using absorbance type measurements and laboratory robots in the analysis of samples to verify the accuracy of the dispensing portion of the robot. This method finds particular use in diagnostic instruments analyzing samples which contain viruses (or antibody to the virus) of infectious diseases. Typical of such infectious diseases are the human immunodeficiency virus Type-1(HIV-1).

BACKGROUND OF THE INVENTION

Various diagnostic instruments are known which are used in the detection of infectious disease agents and antibodies thereto. One such instrument uses a microtiter-plate based enzyme linked immunoabsorbant assay (ELISA). The development of microtiter-plate ELISA methods allows the processing of a large number of samples (typically 96 wells per plate) simultaneously. A common problem in the use of the microtiter-plate based ELISA method is the tracking of sample identity. The wells in a microtiter plate are closely spaced. When a blood sample is manually transferred from a sample tube to one of the closely spaced wells, it can easily be misplaced into a neighboring well.

Several laboratory and robotic systems have been developed for the purpose of processing microtiter plates. These devices are designed to increase laboratory throughput and many of these devices also provide positive sample identification through the use of barcode labels. For example, the Zymate robot (Zymark Corporation, Hopkinton, MA, USA) has been adapted for the processing of the microtiter plates in the research laboratory. Another laboratory automation device that has been in use in may clinical laboratories is the Hamilton MICROLAB-AT (Hamilton Corporation, Reno, Nevada, USA).

The Hamilton MICROLAB-AT, which is also supplied by DuPont as an OEM equipment known as the SUMMIT(TM), is capable of performing the following functions:

1) Reading the barcode labels on each of the 96 sample tubes placed in a tube rack.
2) Adding a precisely measured amount of reagent to each of the 96 wells on the microtiter plate.
3) Transferring a precisely measured amount of sample from the sample tube to the corresponding well on the microtiter plate.
4) Mixing the reagent with the sample and performing any dilution, if required.

In order to minimize the hazards involved in the transfer of human serum which may contain infectious disease agents, the DuPont SUMMIT uses disposable pipets which are ejected into a biohazardous bag after each run. The use of disposable pipets, however, makes the verification of sample accuracy and precision more difficult.

The relatively small size of the wells on the microtiter plate (typically 300 uL per well) requires the precise delivery of a minute amount of sample (typically 10 uL or less). Inaccuracies in the delivery of samples will lead to erroneous results that may endanger public health and the safety of blood supplies. In order to safeguard the accuracy of the ELISA results, the volume delivered by the automated devices must be routinely verified.

Typically, the verification of the volumetric accuracy of a device is accomplished by weighing a sample of pure water transferred by the device. Although this method is a satisfactory procedure for the verification of the delivery of relatively large sample sizes (100 uL or more, ie, 0.1 g in weight), it is not a satisfactory method for the verification of volumes in the range of 5-10 uL. Sample sizes in the range of 5-10 uL are often required for many microtiter-plate based ELISA methods.

When attempts are made to weigh a 10 uL (or 0.01 g) sample of pure water, the surface tension of water will often lead to the incomplete transfer of the liquid from the pipet tip to the microtiter plate. Moreover, evaporative loss will often lead to errors in the measurement. In order to prevent evaporative loss, the relative humidity of the room must be kept very high. In addition, a highly accurate analytical balance must be used for this kind of measurement.

The proper type of equipment and operating environment which are needed to make gravimetric verification of automated pipetting devices are usually not available in a clinical laboratory. Even if these equipment were available, the skill level and time required to conduct these measurements are very high. Thus, the gravimetric verification of the volumetric accuracy of the robotic ELISA processing devices is not

practical in a clinical laboratory.

Typically, in the prior art, colorimetric reagents have been used to verify the volume delivered by a liquid transfer device. In these cases the volume of the reagent is usually calculated by the following equation. (Beers Law):

$$A = a\,b\,c$$

where A=absorbance measured
a=molar absorptivity
b=path length (usually fixed for a given instrument)
c=concentration of colorimetric reagent

Using this common approach, the measured volume is dependent on the absolute concentration of the reagent. Therefore, the approach requires that the exact concentration of the reagent is known.

In reality the concentration of the colorimetric reagent, cannot be precisely controlled. Therefore, verification methods that calculate volume directly from the absorbance measured are often inaccurate. Another verification method is justified in U.S. Patent 4,354,376 issued to Greenfield et al. According to Greenfield et al, "in carrying out the invention, a kit is provided comprising a vial of a standard calibration solution of a first predetermined color density, a vial of a standard calibration solution of a third predetermined color density, a calibration vial containing a predetermined volume of a diluent, and one or more vials or bottles, each containing a solution of a calibration reagent of a different color density. The kit may also contain a pipette calibration chart on which spectrophotometer readings of the light adsorption by the solutions in the standard calibration vials are plotted to provide a reference curve can be used to determine the volume of the calibration reagent pipetted into a calibration vial to replace an equal volume of diluent removed therefrom. That volume is the volume of the pipette being calibrated".

The difficulty with Greenfield et al is a manual procedure apparently used for the occasional pipette that needs to be calibrated. It is not at all suitable for a method that contemplates calibrating an automated device.

SUMMARY OF THE INVENTION

Many of the disadvantages of the prior art dispenser verification systems are overcome by the method of this invention. According to this invention, a method of determining the accuracy of a liquid dispensing pipet using a polarity of wells of the same configuration and volume is accomplished by the steps of: introducing a known volume of a colorimetric reagent that absorbs radiant energy in a manner directly proportional to the concentration into a first well, dispensing a desired volume of the colorimetric reagent using the liquid dispensing pipette into a second one of the wells, measuring the absorbance of the reagent in each of the wells, calculating actual volume of reagent dispensed in the second well by multiplying the known volume by the ratio of the absorbance of reagent in the second well to the absorbance of the reagent in the first well.

In a preferred embodiment the calculated actual volume as compared to the desired volume furthermore the wells are disposed in rows and columns and the known volume of colorimetric reagent introduced into each well of the first two of wells, the absorbance of each well of the first row is averaged to obtain an average absorbance, and the actual volume is calculated using the average absorbance of the first row of wells.

In a preferred embodiment of the invention the colorimetric reagent is cobalt sulfate. In the further embodiment of the method of this invention additional rows of wells wherein additional rows of wells are each dispensed with the desired volume of different concentration of the colorimetric reagent calculating the actual volume of each of the different desired volumes dispensed by multiplying the known volume by the ratio of the absorbance of reagent in each dispensed well to the average absorbance of the first row of wells.

By the use of this invention and its unique way of measuring the volume delivered the need for a colorimetric reagent with a precisely known absorbance value is climinated. Instead of calculating the volume delivered directly from a measured absorbance value, the volume is calculated from a ratio of the absorbance values for a sample delivered by a precisely calibrated pipet and a sample delivered by the automated device thus the error introduced by the variations in concentrations of the reagent is significantly

reduced.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention may be more fully understood from the following detailed description thereof taken in connection with accompanying drawings which form a part of this application and in which:

Figure 1 is a pictorial view of a SUMMIT™ sample transfer system having a robotic arm for effecting the transfer of samples and reagents to microtiter plates for reaction and measurement.

Figure 2 is a fragmentary pictorial view of the robotic arm of Figure 1.

Figure 3 is a block diagram depicting the interaction of the software, used in the method of the inventor, with SUMMIT™ instrument; describes the equipment utilized to perform the method of this invention and

Figures 4a -4i are flow charts of the software used to implement the method of this invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT OF THE METHOD

The method of this invention preferably is used with the SUMMIT™ Automated Transfer System sold by E.I. Du Pont de Nemours and Company, Wilmington, Delaware and the invention will be described in that context. The SUMMIT™ system includes a robotic type instrument 10 (Figs 1 and 2) in which a robotic arm 12 transfers selected volumes of liquid (reagents, diluents and samples) to wells 14 in microtiter plates 16. The wells 14 are typically arranged in rows and columns to form typically an 8 x 12 assay of wells 14. The samples to be processed are held in a certain rack location 18. An array of pipettes 20 mounted on the robtic arm 12 can withdraw samples from the sample locations and transfer them to the wells 14 of microtiter plates 16. Adjacent to the microtiter plates 14 (microplate) are reagent and diluent reservoirs 22.

Referring to Figure 3 it is seen that the SUMMIT™ instrument includes verification software 24 which operating in conjunction with a microplate reader 26 measures the absorbance of a colorimetric reagent of different concentrations in each of the wells of the microplate 14. For this purpose a colorimetric reagent is used. Such colorimetric reagent may be any of those stable reagents well known in the art that follow Beer's Law. These include cobalt sulfate and potassium dichromate, with cobalt sulfate being preferred. When cobalt sulfate is used, sulfuric acid is used as a diluent to maintain stability of the reagent. The software performs a routine in which the pipetting arms 20 dispense different concentrations of the reagent in the several wells 14 (usually row by row), the concentration of each well is measured, and result printed out in the form of reports at the printer 22 stating the accuracy of the dispensed material.

In accordance with the invention, instead of calculating the volume delivered directly from the measured absorbance value, the volume is calculated from a ratio of the absorbance values for a sample delivered by a precisely calibrated pipette and a sample delivered by the automated device. Thus, the error introduced by variations in the concentration of the reagent is eliminated. Using this common approach, the measured volume is dependent on the absolute concentration of the reagent. Therefore, the approach requires that the exact concentration of the reagent is known.

A preferred method to prepare a reference solution "knowing" the exact concentration is containing the colorimetric reagent which preferably is calibrated by a gravimetric method. The gravimetric method includes determining the volume based on weight measurements. The actual volume (V) delivered (in uL) equals:

V = 1000 [uL/mL) x weight (g) x volume factor (mL/g) (at the appropriate temperature)

The volume factor is the volume of 1.000g of pure water at the given temperature.

For Example:

The volume factor at 23° C is 1.0035 mL/g. If the weight of the water delivered is equal to 0.10001g at 23° C, the volume delivered is:

V = 1000 uL/mL x 0.10001g x 1.0035 mL/g = 100.36 uL

The calibrated volume of the reference pipette should be calculated from the average of at least ten weight measurements using a calibrated analytical balance. This reference volume typically is introduced manually into each well 14 of a row of one of the micro-plates, which is typically twelve wells.

The instrument control software as will be described instructs the instrument to fill the micro-plate as follows:

a) Dispensing of Reagent for Blank-Absorbance Correction The first row of wells (A1-A12) (see table below) will be filled with 1% sulfuric acid and will be used to calculate the blank absorbance. The blank absorbance is the average of wells A1-A12.

b) Volume and Calculations To measure the volume dispensed at 10uL, the second row of wells (B1-B12) are first filled with 90 uL of 1% sulfuric acid. Then 10 uL of the Cobalt Sulfate reagent are dispensed into the 1% sulfuric acid and the two reagents are mixed by moving the plunger of the pipet dispenser (included in robotic arm-12) up and down several times.

This mode of dispensing ensures that the colorimetric reagent is completely dispensed. When attempts are made to dispense a 10-uL droplet of liquid onto a "dry" microtiter plate. the surface tension on the droplet will cause the liquid to adhere to the tip of the pipet and consequently prevent a complete transfer of the reagent.

To ensure that volumes other than 10 uL are also accurately dispensed, the rest of the microtiter plate is filled with different volumes of the colorimetric reagent Cobalt Sulfate and its diluent 1% sulfuric acid as summarized below:

Row	uL H_2SO_4	uL $CoSO_4$	Comment
A1-A12	100 uL	0 uL	Blank by SUMMIT
B1-B12	90	10	dispensed by SUMMIT
C1-C12	80	20	dispensed by SUMMIT
D1-D12	70	30	dispensed by SUMMIT
E1-E12	60	40	dispensed by SUMMIT
F1-F12	50	50	dispensed by SUMMIT
G1-G12	0	100	dispensed by SUMMIT
H1-H12	0	100	by reference pipette

The software assumes that

i) The volume pipetted into wells 111-1112 is correct. These wells are pipetted manually using a calibrated pipet.

ii) The absorbance (A) of Cobalt Sulfate, in 1% sulfuric acid, is directly proportional to its concentration (Beer's Law). Therefore, the volume of Cobalt Sulfate solution (V) delivered to each well is calculated as:

V = (Absorbance measured/Av Absorbance 111-1112) x Volume of Calibrated Pipette

The software also allows for the correction of the error in the reference pipet. The calculated volume is compared with the gravimetrically calibrated volume instead of the nominal volume of the reference pipet as shown in the above equation. To obtain absorbance measurement of the cobalt sulfate reagent, the filled microtiter plate is read at 492 nM. Software of this invention performs all the necessary data collection and calculations. A report is generated and a sample report is attached. The details of the software may be best understood from the flow chart of Figure 3.

SYSTEM SOFTWARE

The IBM Computer (IBM AT or IBM PS2/50) interfaces with the SUMMIT (TM)and an automatic microplate reader through two separate serial interface ports.

The software program sends command sequences to the SUMMIT controller electronics, effectuates the pipetting of samples and receives absorbance data from the microplate reader.

SYSTEM OPERATION

To begin the verification process, the user will access the software program and select the "Volumes Verification Menu" (Figure 2.a). Normally the user will then select the "Start Volumes Verification" option.

The software program is interactive and instructs the user to perform certain tasks during the verification process. For example, the software program will instruct the user to place the appropriate reagents at the proper locations by showing a depiction of the SUMMIT (TM) reagent rack (Figure 2.b). If the user wishes to stop the procedure, he may choose the "cancel" option at any time. The junctions for the "cancel" options are shown in Figures 2.b to 2.g.

After the reagents are placed in the correct locations, the user will continue the verification process by selecting the "Fill Verification Plate" option. A series of commands will be sent to the SUMMIT and strips A through G will be filled automatically.

When rows of wells A through G are completely filled, the SUMMIT instrument will stop and the software program will tell the user to fill strip H with a calibrated reference pipet. Once this step is completed, the user will place the microtiter plate into the microtiter reader by selecting the "Read Verification Plate" option. Data collection will be performed automatically and the data will be displayed on the screen. If an error occurs during the data collection step, the user has the option to repeat the data collection process by selecting the "Reread Verification Plate" option (Figure 2.c). Various calculations will be performed and the user has the option to print a summary report or a full report with graphs. Data will be saved automatically on the system hard-disk and can be recalled anytime by selecting the "View Old Verification Plate" option (Figure 2.d).

For record keeping purposes, the user will store the serial number of the SUMMIT, the reagent lot number and the reagent expiration date in the system by using the "View/Edit Verification Reagent Data" option (Figure 2.e).

INSTRUMENT CONTROL AND CALCULATIONS

Details for the instrument control functions of the software program are shown in Figures 2.f and 2.g. During the "Initialize SUMMIT" step, commands will be sent to the SUMMIT to find the starting position for the stepper motors. Twelve disposable pipet tips will then be loaded and the plate will be filled automatically. The user may choose to abort the filling process at anytime by selecting the cancel option. Details of the filling procedure for individual strips are shown in Figure 2.g. During the process of filling each strip, the software program will instruct the SUMMIT to aspirate the reagent, wipe the tips and pipette the reagent into the microtiter plate. The amounts of cobalt sulfate and sulfuric acid required for each strip are shown in Figure 2.f. Details of the calculation algorithm and the report printing format are shown on Figures 2.h and 2.i, respectively.

The method described is a relatively simple yet highly accurate means for determining the accuracy of the automatic dispensing pipets. It does not rely upon the accuracy of a particular reagent but rather relies upon comparisons made to a reference.

Claims

1. A method of determining the accuracy of a liquid dispensing pipet using a plurality of wells of the same configuration and volume by the steps of:
   introducing a known volume of a colorimetric reagent of a known concentration that absorbs radiant energy in a manner directly proportional to its concentration into a first well,
   dispensing a first desired volume of the colorimetric reagent using the liquid dispensing pipet into a second well,
   measuring the absorbance of the reagent in each of the wells, and
   calculating the actual volume of reagent dispensed in the second well by multiplying the know volume by the ratio of the absorbance of reagent in the second well to the absorbance of the reagent in the first well.

2. The method of claim 1 which includes the additional step of comparing the calculated actual volume with the desired volume.

3. The method of claim 3 wherein any stable colorimetric reagent such as cobalt sulfate or potassium dichromate can be used.

4. The method of claim 1 which includes the additional steps of dispensing a second desired volume of the colorimetric reagent together with a first desired volume of a diluent for the reagent into a third well, and calculating the actual volume of reagent dispensed in the third well by multiplying the know volume by the ratio of the absorbance of the reagent in the third well to the absorbance of the reagent in the first well.

5. In the method of claim 4 wherein the diluent is sulfuric acid.

6. In the method of claim 2 which includes the additional steps of dispensing a second desired volume of the colorimetric reagent together with a first desired volume of a diluent for the reagent into a third well, and calculating the actual volume of reagent dispensed in the third well by multiplying the known

volume by the ratio of the absorbance of the reagent in the third well to the absorbance of the reagent in the first well.

7. In the method of claim 6 wherein the colorimetric reagent is cobalt sulfate.

8. In the method of claim 3 wherein additional rows of wells are each dispensed with the desired volume of different concentrations of the colorimetric reagent, calculating the actual volume of each of the different desired volumes dispensed by multiplying the known volume by the ratio of the absorbance of reagent in each dispensed well to the average absorbance of the first row of wells.

Fig. 1

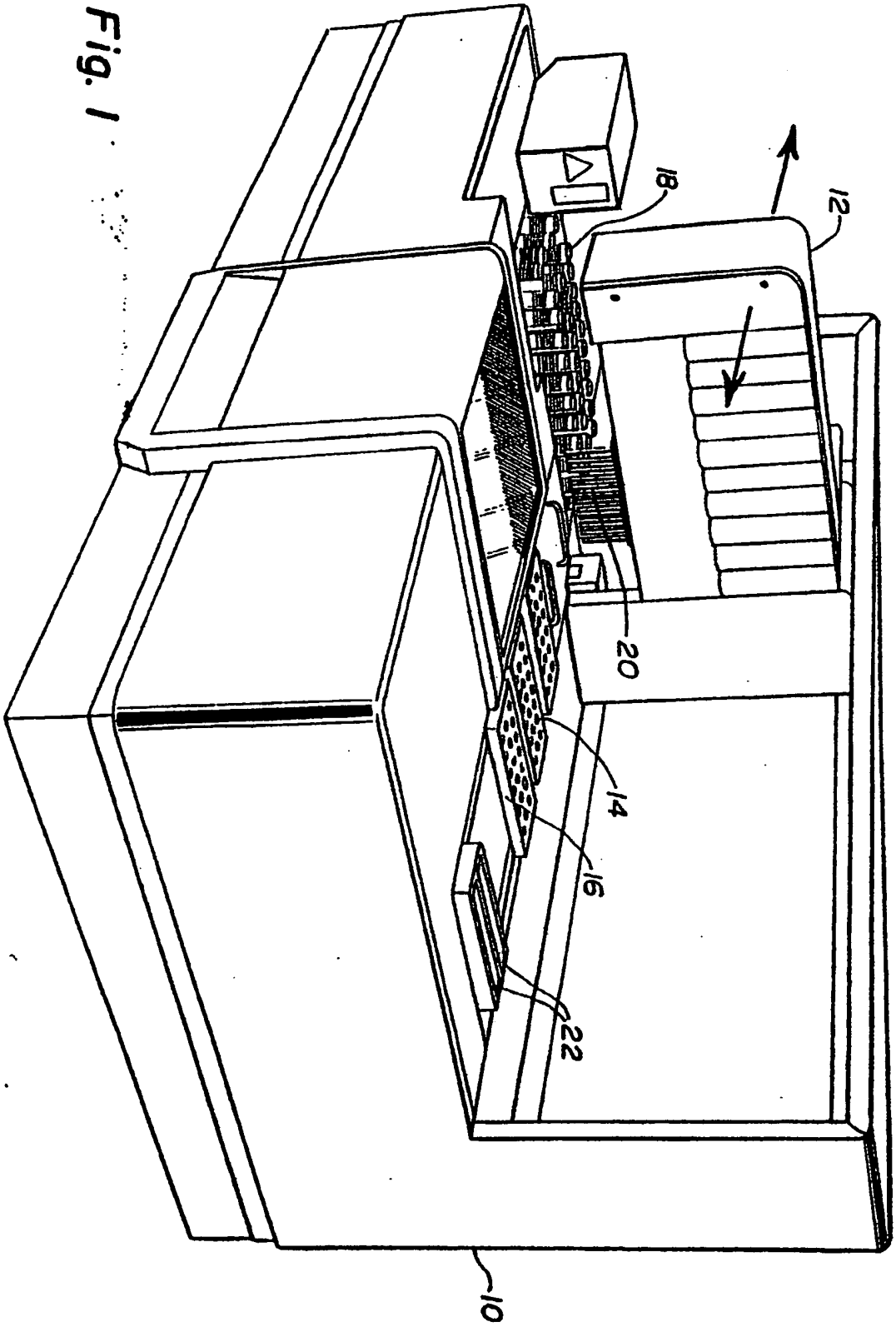
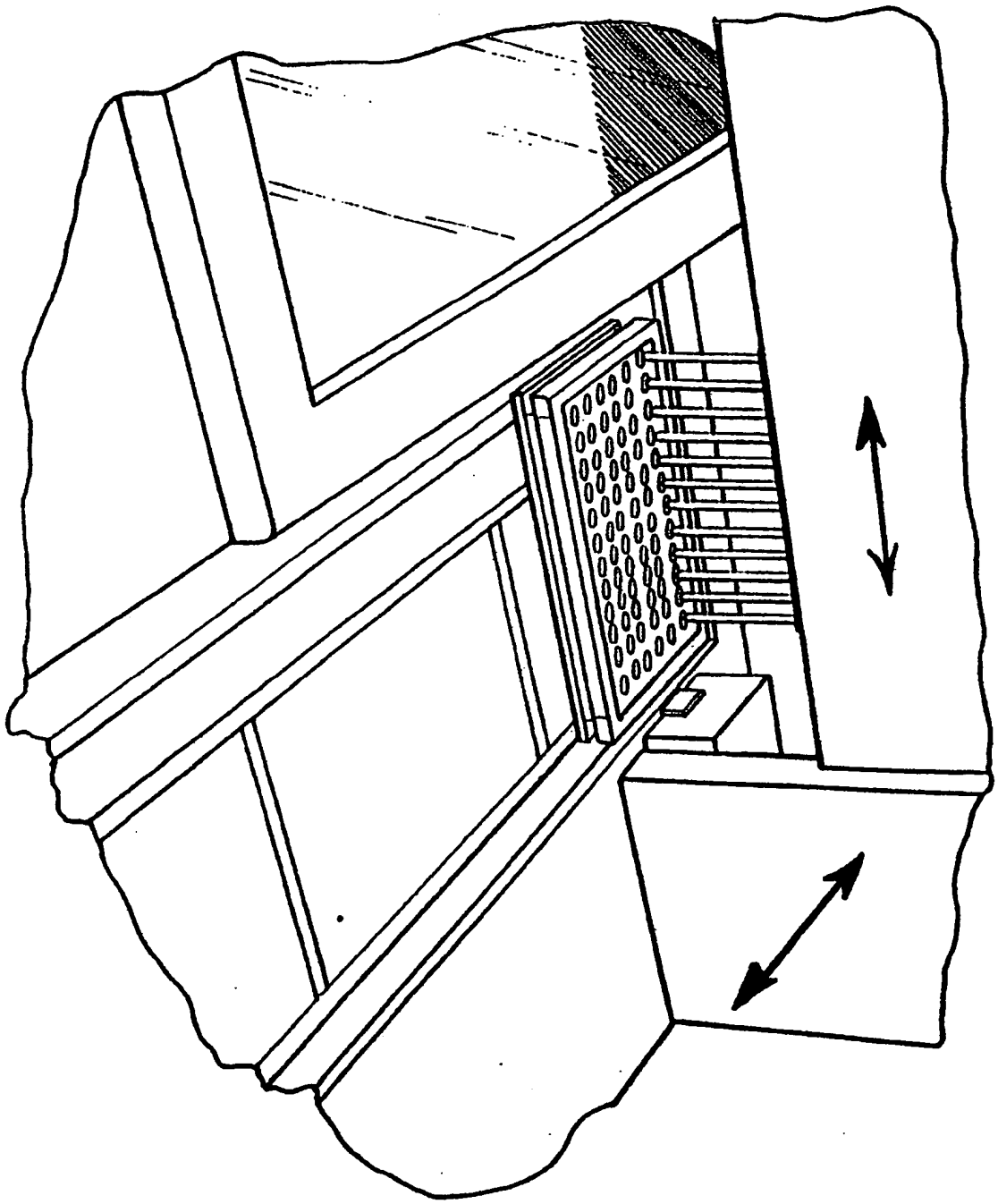


Fig. 2



VERIFICATION SYSTEM LAYOUT

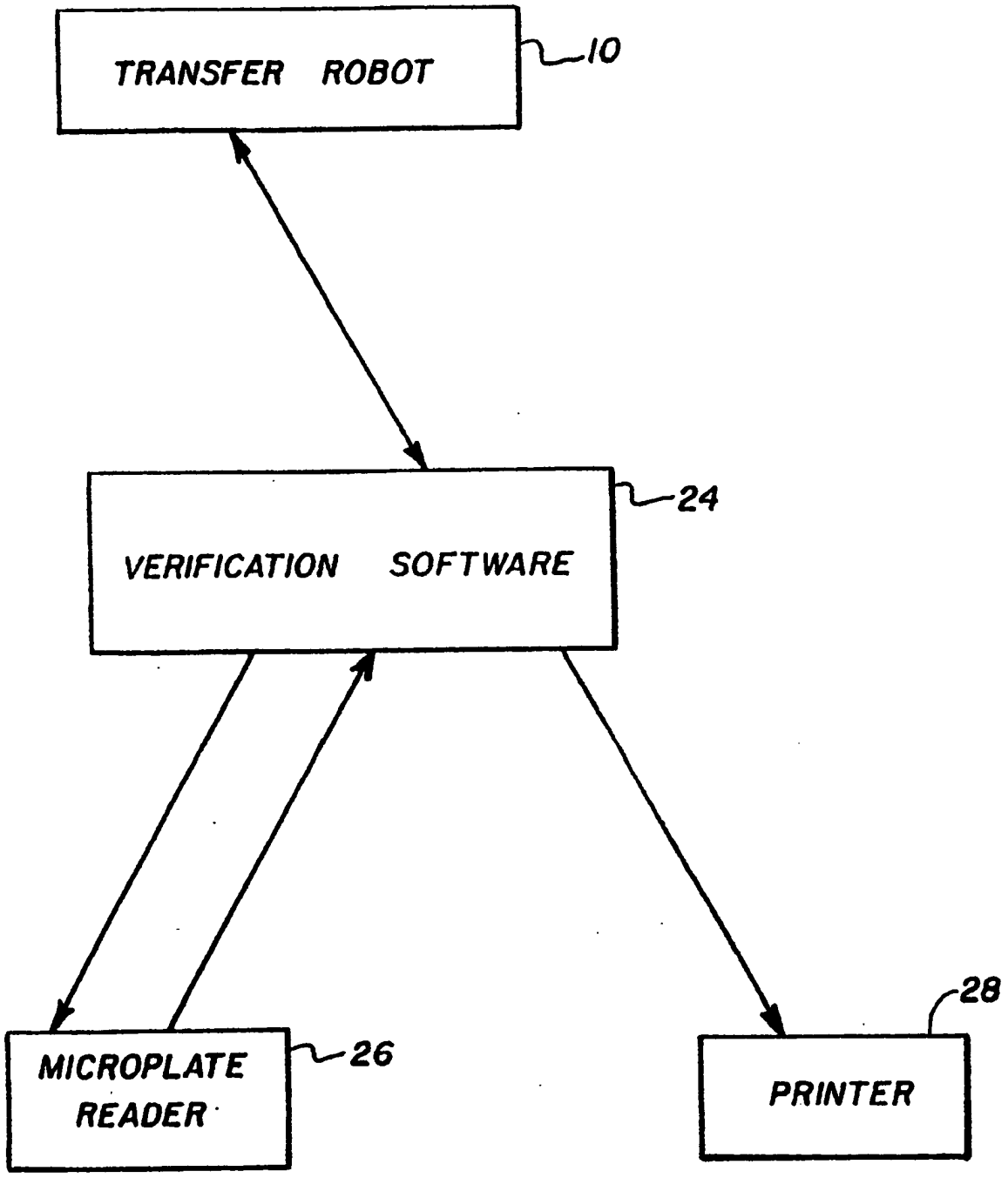


Fig. 3

Fig. 4a

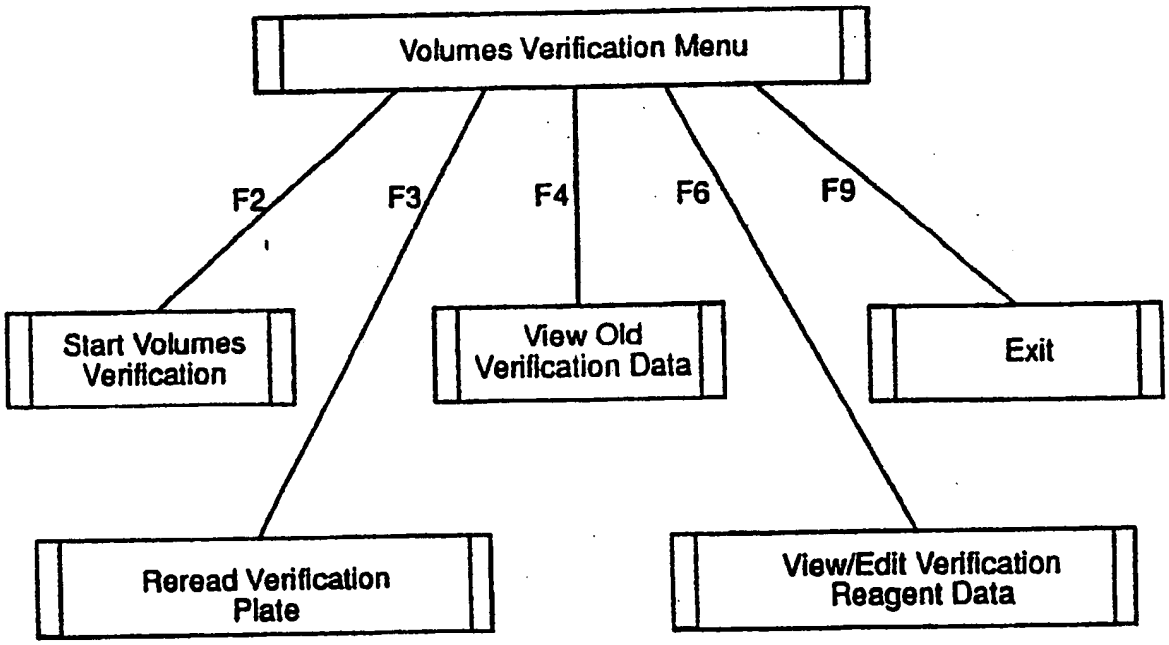
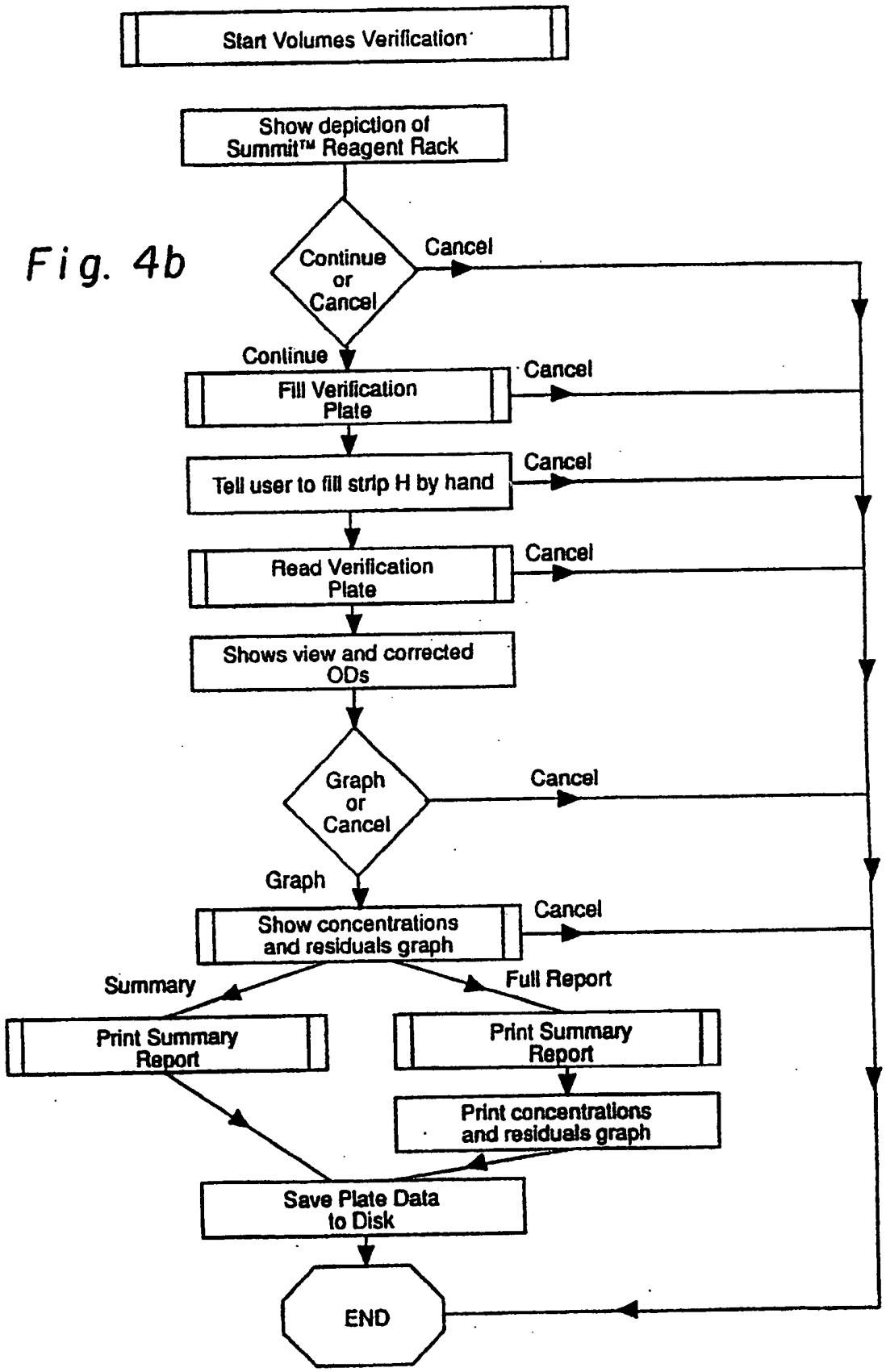
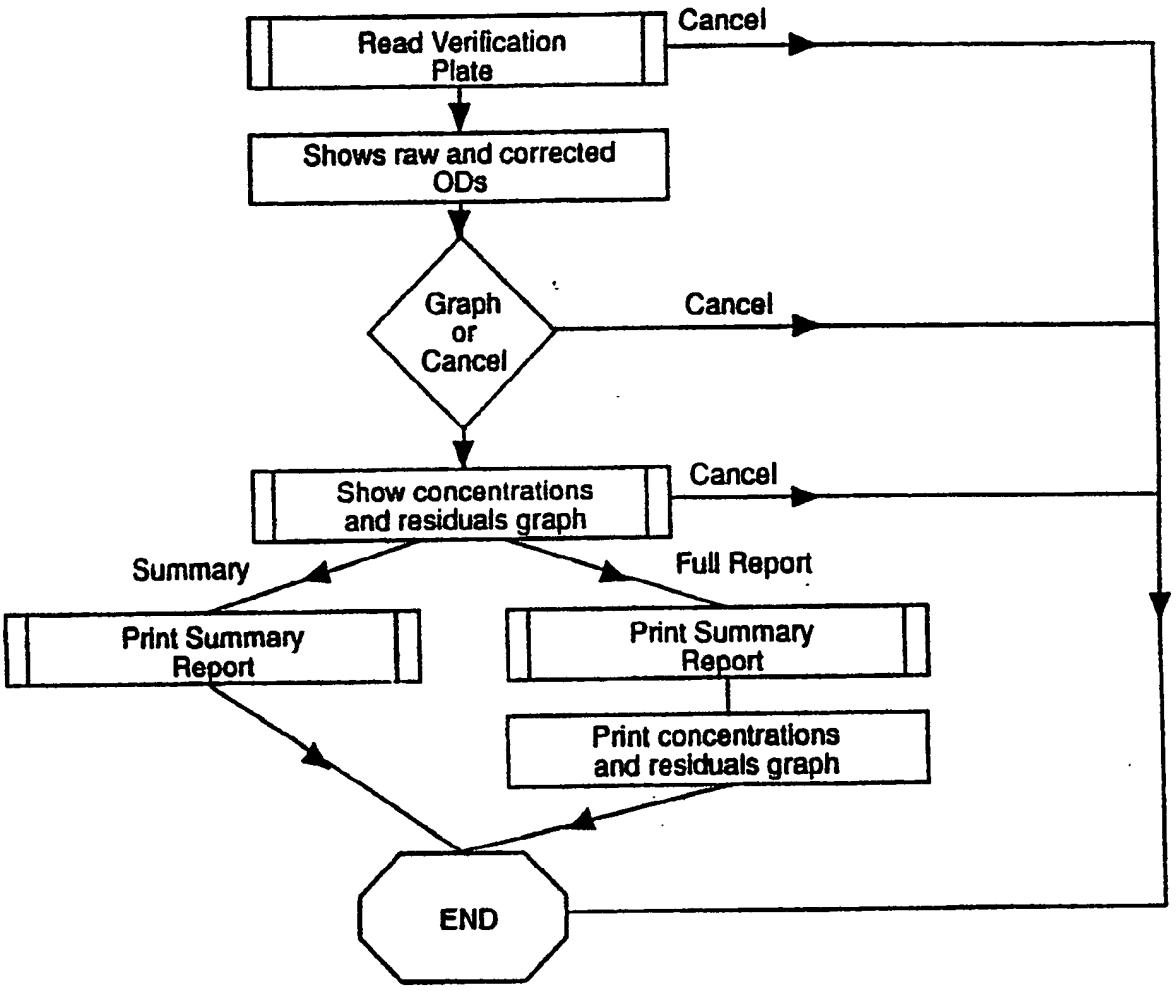

Volumes Verification Menu
F2
F3
F4
F6
F9
Start Volumes Verification
View Old Verification Data
Exit
Reread Verification Plate
View/Edit Verification Reagent Data

Fig. 4b


Start Volumes Verification
Show depiction of Summit™ Reagent Rack
Continue or Cancel
Cancel
Continue
Fill Verification Plate
Cancel
Tell user to fill strip H by hand
Cancel
Read Verification Plate
Cancel
Shows view and corrected ODs
Graph or Cancel
Cancel
Graph
Show concentrations and residuals graph
Cancel
Summary
Full Report
Print Summary Report
Print Summary Report
Print concentrations and residuals graph
Save Plate Data to Disk
END

Reread Verification Plate

Fig. 4c


Read Verification Plate
Cancel
Shows raw and corrected ODs
Graph or Cancel
Cancel
Show concentrations and residuals graph
Cancel
Summary
Full Report
Print Summary Report
Print Summary Report
Print concentrations and residuals graph
END

View Old Verification Plate

Fig. 4d

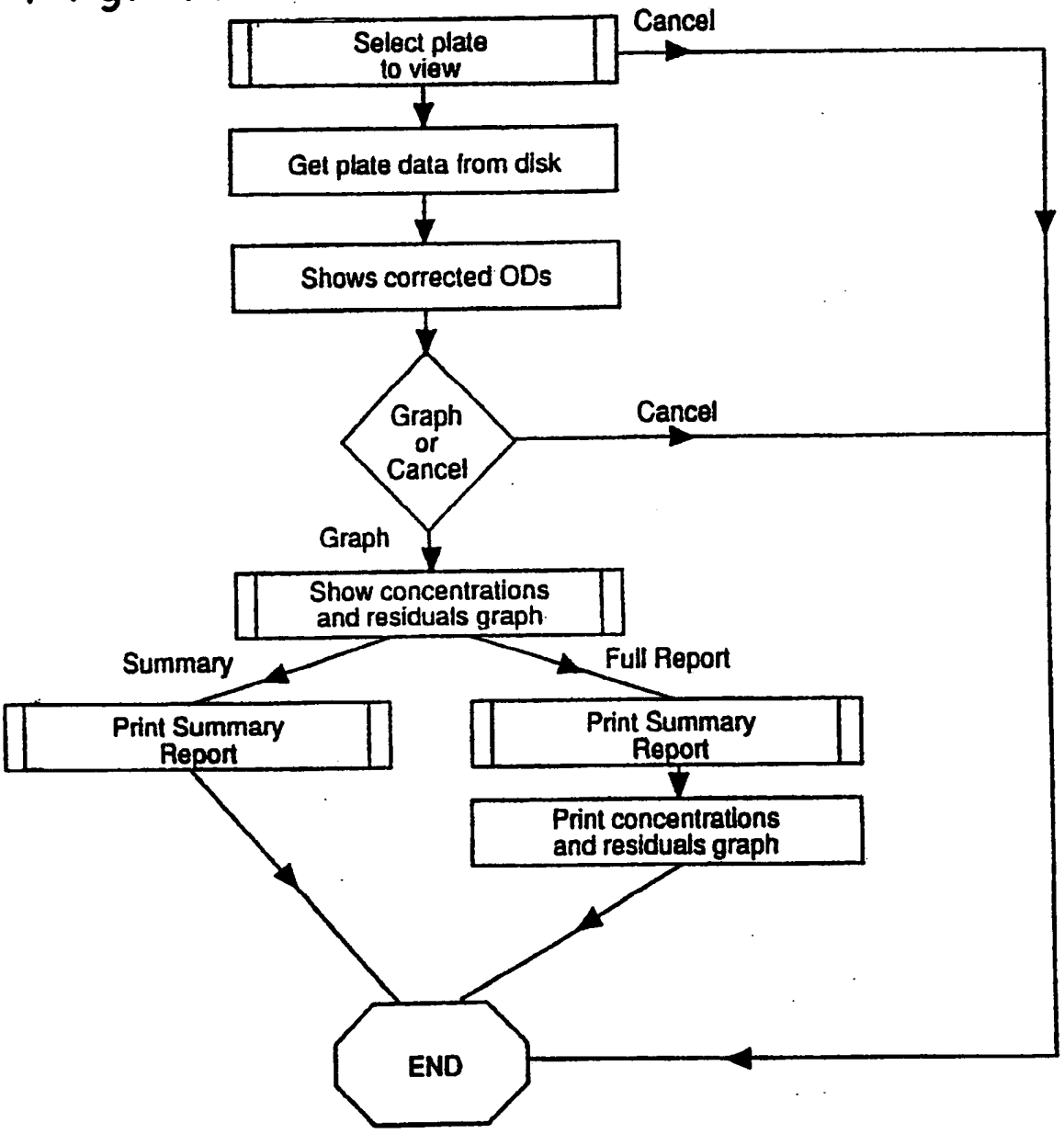
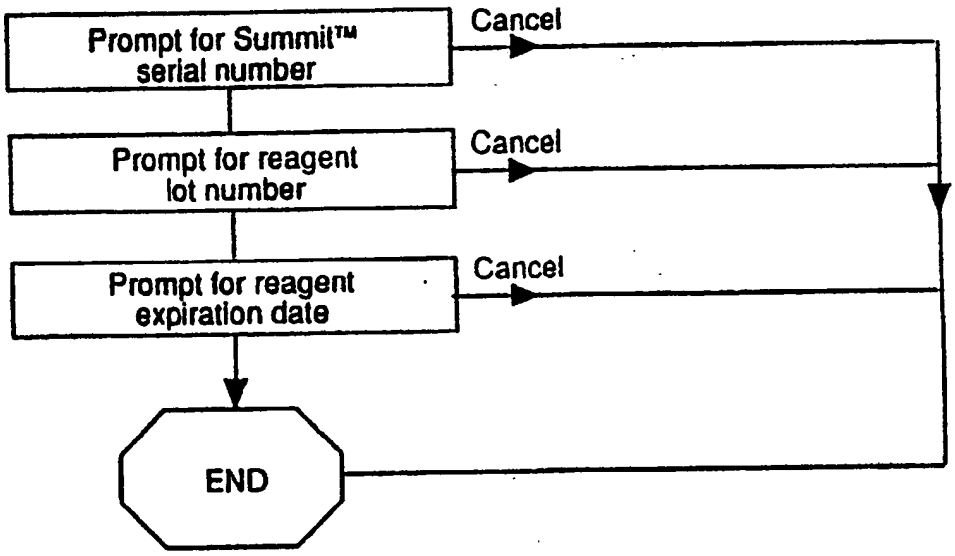

Select plate to view
Cancel
Get plate data from disk
Shows corrected ODs
Graph or Cancel
Cancel
Graph
Show concentrations and residuals graph
Summary
Full Report
Print Summary Report
Print Summary Report
Print concentrations and residuals graph
END

Fig. 4e

View/Edit Verification
Reagent Data


Prompt for Summit™
serial number
Cancel
Prompt for reagent
lot number
Cancel
Prompt for reagent
expiration date
Cancel
END

Fill Verification Plate

Fig. 4f

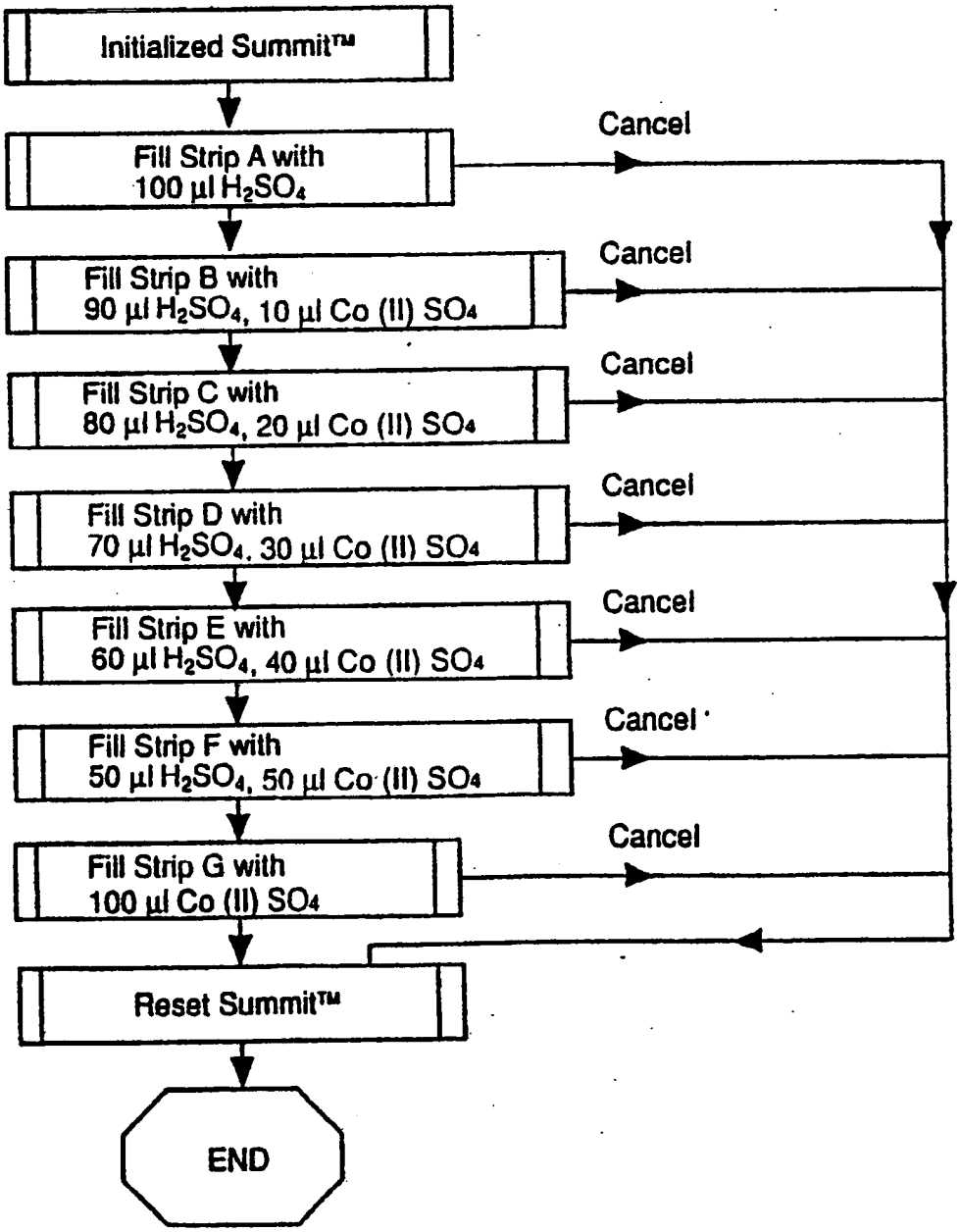

Initialized Summit™
Fill Strip A with 100 µl H_2SO_4
Cancel
Fill Strip B with 90 µl H_2SO_4, 10 µl Co (II) SO_4
Cancel
Fill Strip C with 80 µl H_2SO_4, 20 µl Co (II) SO_4
Cancel
Fill Strip D with 70 µl H_2SO_4, 30 µl Co (II) SO_4
Cancel
Fill Strip E with 60 µl H_2SO_4, 40 µl Co (II) SO_4
Cancel
Fill Strip F with 50 µl H_2SO_4, 50 µl Co (II) SO_4
Cancel
Fill Strip G with 100 µl Co (II) SO_4
Cancel
Reset Summit™
END

Fig. 4g

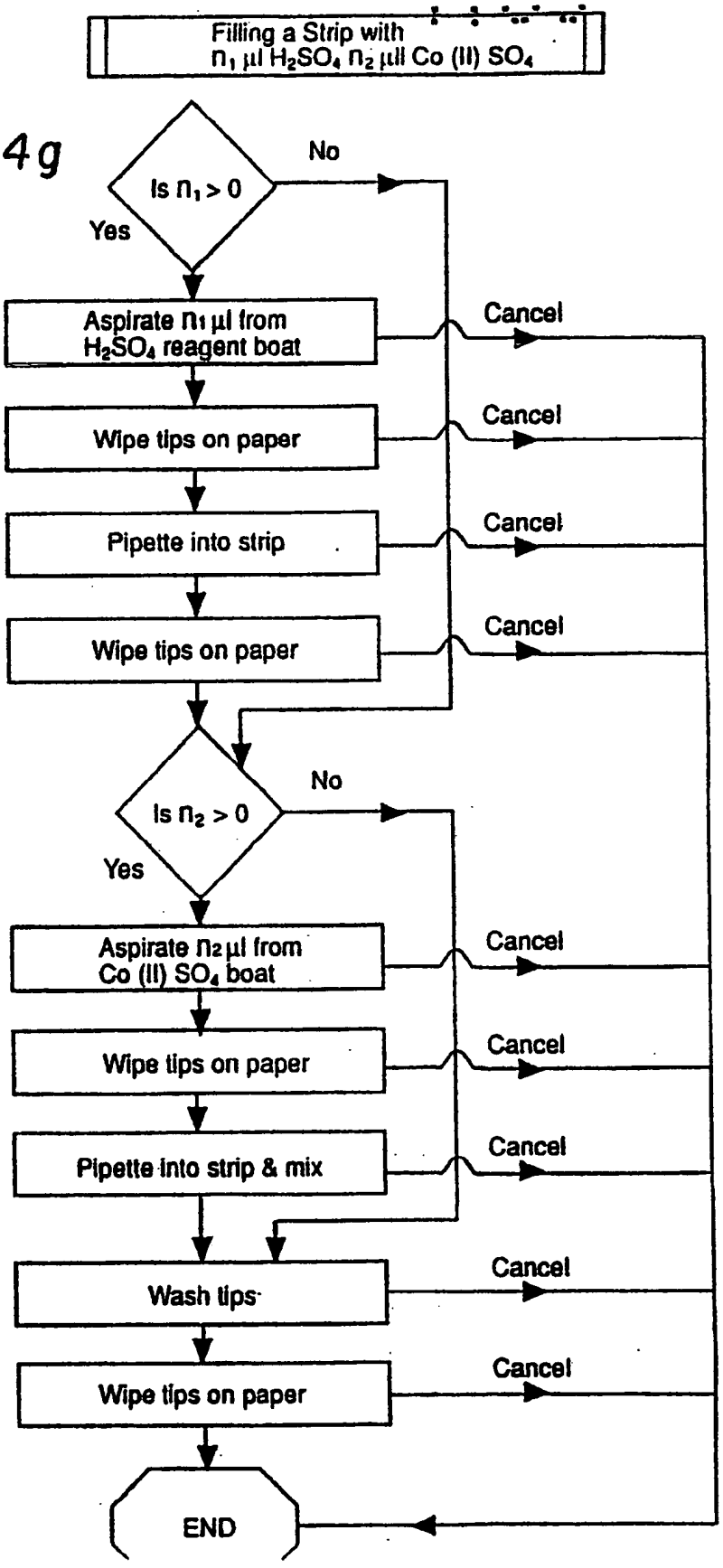

Filling a Strip with
n1 µl H2SO4 n2 µll Co (II) SO4
Is n1 > 0
No
Yes
Aspirate n1 µl from
H2SO4 reagent boat
Cancel
Wipe tips on paper
Cancel
Pipette into strip
Cancel
Wipe tips on paper
Cancel
Is n2 > 0
No
Yes
Aspirate n2 µl from
Co (II) SO4 boat
Cancel
Wipe tips on paper
Cancel
Pipette into strip & mix
Cancel
Wash tips
Cancel
Wipe tips on paper
Cancel
END

Fig. 4h

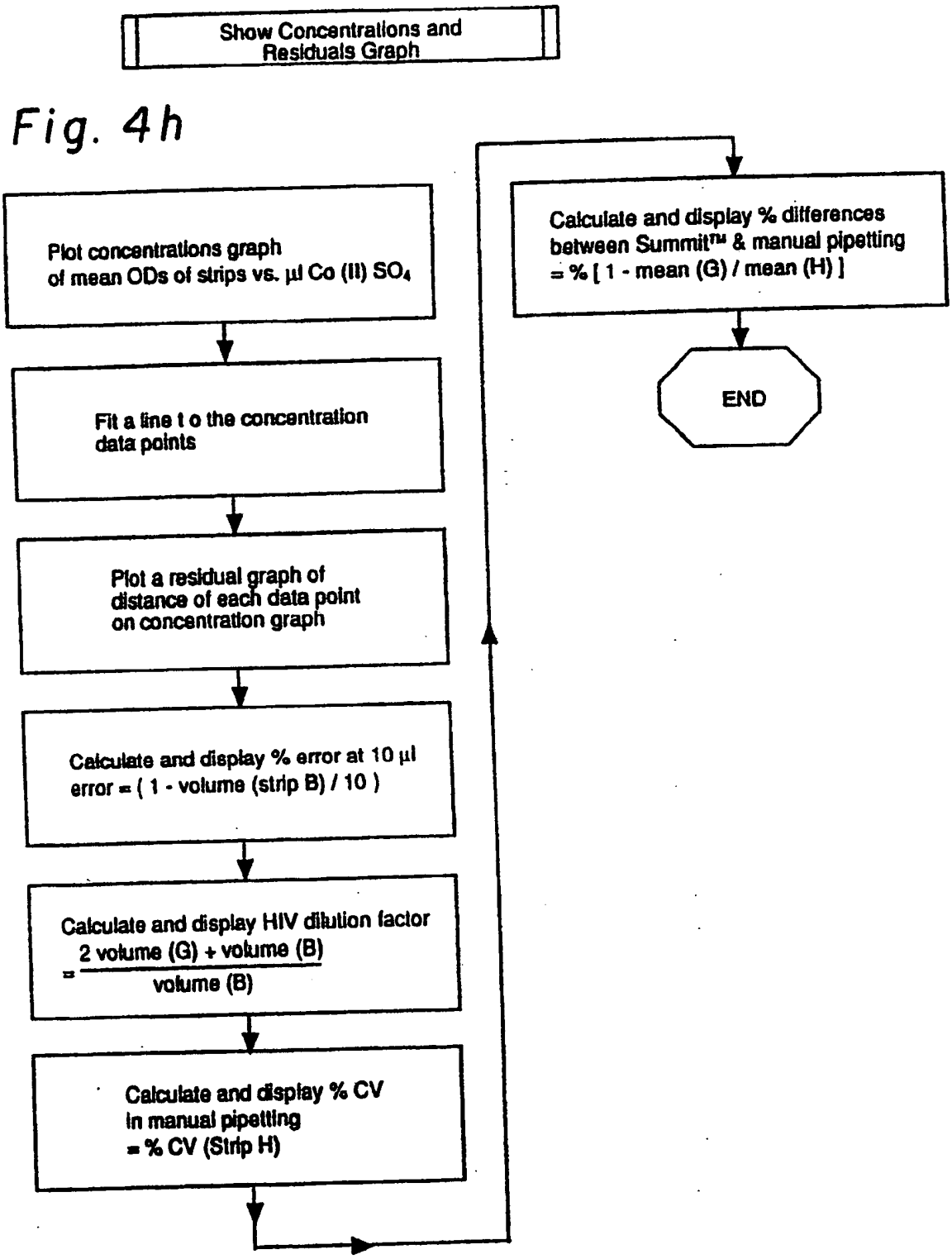
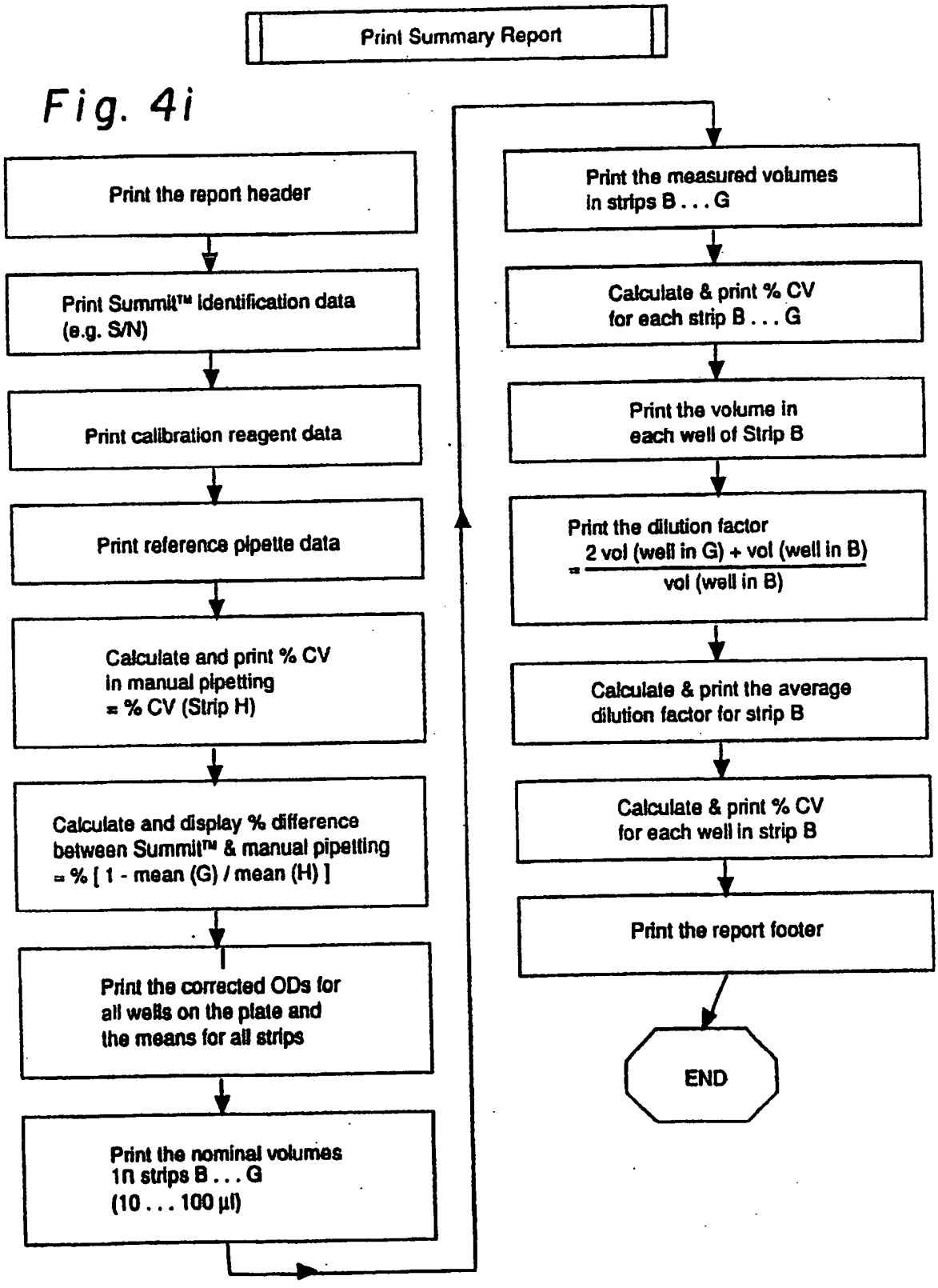

Show Concentrations and Residuals Graph
Plot concentrations graph of mean ODs of strips vs. µl Co (II) SO_4
Fit a line t o the concentration data points
Plot a residual graph of distance of each data point on concentration graph
Calculate and display % error at 10 µl
error = (1 - volume (strip B) / 10)
Calculate and display HIV dilution factor
= 2 volume (G) + volume (B) / volume (B)
Calculate and display % CV in manual pipetting
= % CV (Strip H)
Calculate and display % differences between Summit™ & manual pipetting
= % [1 - mean (G) / mean (H)]
END

Fig. 4i


Print Summary Report
Print the report header
Print Summit™ identification data (e.g. S/N)
Print calibration reagent data
Print reference pipette data
Calculate and print % CV in manual pipetting = % CV (Strip H)
Calculate and display % difference between Summit™ & manual pipetting = % [1 - mean (G) / mean (H)]
Print the corrected ODs for all wells on the plate and the means for all strips
Print the nominal volumes in strips B . . . G (10 . . . 100 µl)
Print the measured volumes in strips B . . . G
Calculate & print % CV for each strip B . . . G
Print the volume in each well of Strip B
Print the dilution factor = 2 vol (well in G) + vol (well in B) / vol (well in B)
Calculate & print the average dilution factor for strip B
Calculate & print % CV for each well in strip B
Print the report footer
END

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EP-A- 0 374 686
US-A- 4 354 376
US-A- 4 586 546
US-A- 4 772 487

(73) Proprietor : **E.I. DU PONT DE NEMOURS AND COMPANY**
1007 Market Street
Wilmington Delaware 19898 (US)

(72) Inventor : **Lung, Kaki Rick**
48 Quail Hollow Drive
Hockessin, Delaware 19707 (US)
Inventor : **Fok, John Shiu-Kang**
41 Slashpine Circle
Hockessin, Delaware 19707 (US)
Inventor : **Chanin, Alex Joseph**
8837 Rising Sun Avenue
Philadelphia, Pennsylvania 19115 (US)

(74) Representative : **von Kreisler, Alek, Dipl.-Chem. et al**
Patentanwälte
von Kreisler-Selting-Werner
Postfach 10 22 41
D-50462 Köln (DE)

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Description

FIELD OF THE INVENTION

This invention relates to a method of determining the accuracy of a liquid dispenser. More particularly, this invention relates to a method of use of robotic equipment in the processing of microtiter-plate based assays. The method permits diagnostic instruments using absorbance type measurements and laboratory robots in the analysis of samples to verify the accuracy of the dispensing portion of the robot. This method finds particular use in diagnostic instruments analyzing samples which contain viruses (or antibody to the virus) of infectious diseases. Typical of such infectious diseases are the human immunodeficiency virus Type-1(HIV-1).

BACKGROUND OF THE INVENTION

Various diagnostic instruments are known which are used in the detection of infectious disease agents and antibodies thereto. One such instrument uses a microtiter-plate based enzyme linked immunoabsorbant assay (ELISA). The development of microtiter-plate ELISA methods allows the processing of a large number of samples (typically 96 wells per plate) simultaneously. A common problem in the use of the microtiter-plate based ELISA method is the tracking of sample identity. The wells in a microtiter plate are closely spaced. When a blood sample is manually transferred from a sample tube to one of the closely spaced wells, it can easily be misplaced into a neighboring well.

Several laboratory and robotic systems have been developed for the purpose of processing microtiter plates. These devices are designed to increase laboratory throughput and many of these devices also provide positive sample identification through the use of barcode labels.

For example, the Zymate robot (Zymark Corporation, Hopkinton, MA, USA) has been adapted for the processing of the microtiter plates in the research laboratory. Another laboratory automation device that has been in use in many clinical laboratories is the Hamilton MICROLAB-AT (Hamilton Corporation, Reno, Nevada, USA).

The Hamilton MICROLAB-AT, which is also supplied by DuPont as an OEM equipment known as the SUMMIT(TM), is capable of performing the following functions:

1) Reading the barcode labels on each of the 96 sample tubes placed in a tube rack.
2) Adding a precisely measured amount of reagent to each of the 96 wells on the microtiter plate.
3) Transferring a precisely measured amount of sample from the sample tube to the corresponding well on the microtiter plate.
4) Mixing the reagent with the sample and performing any dilution, if required.

In order to minimize the hazards involved in the transfer of human serum which may contain infectious disease agents, the DuPont SUMMIT uses disposable pipets which are ejected into a biohazardous bag after each run. The use of disposable pipets, however, makes the verification of sample accuracy and precision more difficult.

The relatively small size of the wells on the microtiter plate (typically 300 uL per well) requires the precise delivery of a minute amount of sample (typically 10 uL or less). Inaccuracies in the delivery of samples will lead to erroneous results that may endanger public health and the safety of blood supplies. In order to safeguard the accuracy of the ELISA results, the volume delivered by the automated devices must be routinely verified.

Typically, the verification of the volumetric accuracy of a device is accomplished by weighing a sample of pure water transferred by the device. Although this method is a satisfactory procedure for the verification of the delivery of relatively large sample sizes (100 uL or more, ie. 0.1 g in weight), it is not a satisfactory method for the verification of volumes in the range of 5-10 uL. Sample sizes in the range of 5-10 uL are often required for many microtiter-plate based ELISA methods.

When attempts are made to weigh a 10 uL (or 0.01 g) sample of pure water, the surface tension of water will often lead to the incomplete transfer of the liquid from the pipet tip to the microtiter plate. Moreover, evaporative loss will often lead to errors in the measurement. In order to prevent evaporative loss, the relative humidity of the room must be kept very high. In addition, a highly accurate analytical balance must be used for this kind of measurement.

The proper type of equipment and operating environment which are needed to make gravimetric verification of automated pipetting devices are usually not available in a clinical laboratory. Even if these equipment were available, the skill level and time required to conduct these measurements are very high. Thus, the gravimetric verification of the volumetric accuracy of the robotic ELISA processing devices is not practical in a clinical laboratory.

Typically, in the prior art, colorimetric reagents have been used to verify the volume delivered by a liquid transfer device. In these cases the volume of the reagent is usually calculated by the following equation. (Beers Law):

$$A = a\,b\,c$$

where
A=absorbance measured
a=molar absorptivity
b=path length (usually fixed for a given instrument)
c=concentration of colorimetric reagent

Using this common approach, the measured volume is dependent on the absolute concentration of the reagent. Therefore, the approach requires that the exact concentration of the reagent is known.

In reality the concentration of the colorimetric reagent, cannot be precisely controlled. Therefore, verification methods that calculate volume directly from the absorbance measured are often inaccurate. Another verification method is justified in U.S. Patent 4,354,376 issued to Greenfield et al. According to Greenfield et al, "in carrying out the invention, a kit is provided comprising a vial of a standard calibration solution of a first predetermined color density, a vial of a standard calibration solution of a third predetermined color density, a calibration vial containing a predetermined volume of a diluent, and one or more vials or bottles, each containing a solution of a calibration reagent of a different color density. The kit may also contain a pipette calibration chart on which spectrophotometer readings of the light adsorption by the solutions in the standard calibration vials are plotted to provide a reference curve can be used to determine the volume of the calibration reagent pipetted into a calibration vial to replace an equal volume of diluent removed therefrom. That volume is the volume of the pipette being calibrated".

The difficulty with Greenfield et al is a manual procedure apparently used for the occasional pipette that needs to be calibrated. It is not at all suitable for a method that contemplates calibrating an automated device.

SUMMARY OF THE INVENTION

Many of the disadvantages of the prior art dispenser verification systems are overcome by the method of this invention. According to this invention, a method of determining the accuracy of a liquid dispensing pipet using a plurality of wells of the same configuration and volume is accomplished by the steps of: introducing a known volume of a colorimetric reagent that absorbs radiant energy in a manner directly proportional to the concentration into a first well, dispensing a desired volume of the colorimetric reagent using the liquid dispensing pipette into a second one of the wells, measuring the absorbance of the reagent in each of the wells, calculating actual volume of reagent dispensed in the second well by multiplying the known volume by the ratio of the absorbance of reagent in the second well to the absorbance of the reagent in the first well.

In a preferred embodiment the calculated actual volume is compared to the desired volume furthermore the wells are disposed in rows and columns and the known volume of colorimetric reagent introduced into each well of the first two of wells, the absorbance of each well of the first row is averaged to obtain an average absorbance, and the actual volume is calculated using the average absorbance of the first row of wells.

In a preferred embodiment of the invention the colorimetric reagent is cobalt sulfate. In the further embodiment of the method of this invention additional rows of wells wherein additional rows of wells are each dispensed with the desired volume of different concentration of the colorimetric reagent calculating the actual volume of each of the different desired volumes dispensed by multiplying the known volume by the ratio of the absorbance of reagent in each dispensed well to the average absorbance of the first row of wells.

By the use of this invention and its unique way of measuring the volume delivered the need for a colorimetric reagent with a precisely known absorbance value is eliminated. Instead of calculating the volume delivered directly from a measured absorbance value, the volume is calculated from a ratio of the absorbance values for a sample delivered by a precisely calibrated pipet and a sample delivered by the automated device thus the error introduced by the variations in concentrations of the reagent is significantly reduced.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention may be more fully understood from the following detailed description thereof taken in connection with accompanying drawings which form a part of this application and in which:

Figure 1 is a pictorial view of a SUMMIT™ sample transfer system having a robotic arm for effecting the transfer of samples and reagents to microtiter plates for reaction and measurement.

Figure 2 is a fragmentary pictorial view of the robotic arm of Figure 1.

Figure 3 is a block diagram depicting the interaction of the software, used in the method of the inventor,

with SUMMIT™ instrument; describes the equipment utilized to perform the method of this invention; and

Figures 4a - 4i are flow charts of the software used to implement the method of this invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT OF THE METHOD

The method of this invention preferably is used with the SUMMIT™ Automated Transfer System sold by E.I. Du Pont de Nemours and Company, Wilmington, Delaware and the invention will be described in that context. The SUMMIT™ system includes a robotic type instrument 10 (Figs 1 and 2) in which a robotic arm 12 transfers selected volumes of liquid (reagents, diluents and samples) to wells 14 in microtiter plates 16. The wells 14 are typically arranged in rows and columns to form typically an 8x12 assay of wells 14. The samples to be processed are held in a certain rack location 18. An array of pipettes 20 mounted on the robotic arm 12 can withdraw samples from the sample locations and transfer them to the wells 14 of microtiter plates 16. Adjacent to the microtiter plates 14 (microplate) are reagent and diluent reservoirs 22.

Referring to Figure 3 it is seen that the SUMMIT™ instrument includes verification software 24 which operating in conjunction with a microplate reader 26 measures the absorbance of a colorimetric reagent of different concentrations in each of the wells of the microplate 14. For this purpose a colorimetric reagent is used. Such colorimetric reagent may be any of those stable reagents well known in the art that follow Beer's Law. These include cobalt sulfate and potassium dichromate, with cobalt sulfate being preferred. When cobalt sulfate is used, sulfuric acid is used as a diluent to maintain stability of the reagent. The software performs a routine in which the pipetting arms 20 dispense different concentrations of the reagent in the several wells 14 (usually row by row), the concentration of each well is measured, and result printed out in the form of reports at the printer 22 stating the accuracy of the dispensed material.

In accordance with the invention, instead of calculating the volume delivered directly from the measured absorbance value, the volume is calculated from a ratio of the absorbance values for a sample delivered by a precisely calibrated pipette and a sample delivered by the automated device. Thus, the error introduced by variations in the concentration of the reagent is eliminated. Using this common approach, the measured volume is dependent on the absolute concentration of the reagent. Therefore, the approach requires that the exact concentration of the reagent is known.

A preferred method to prepare a reference solution "knowing" the exact concentration is containing the colorimetric reagent which preferably is calibrated by a gravimetric method. The gravimetric method includes determining the volume based on weight measurements. The actual volume (V) delivered (in uL) equals:

$$V = 1000\ (uL/mL) \times \text{weight (g)} \times \text{volume factor (mL/g) (at the appropriate temperature)}$$

The volume factor is the volume of 1.000 g of pure water at that given temperature.

For Example:

The volume factor at 23°C is 1.0035 mL/g. If the weight of the water delivered is equal to 0.10001 g at 23°C, the volume delivered is:

$$V = 1000\ uL/mL \times 0.10001\ g \times 1.0035\ mL/g = 100.36\ uL$$

The calibrated volume of the reference pipette should be calculated from the average of at least ten weight measurements using a calibrated analytical balance. This reference volume typically is introduced manually into each well 14 of a row of one of the micro-plates, which is typically twelve wells.

The instrument control software as will be described instructs the instrument to fill the micro-plate as follows:

a) Dispensing of Reagent for Blank-Absorbance Correction

The first row of wells (A1-A12) (see table below) will be filled with 1% sulfuric acid and will be used to calculate the blank absorbance. The blank absorbance is the average of wells A1-A12.

b) Volume and Calculations

To measure the volume dispensed at 10 uL, the second row of wells (B1-B12) are first filled with 90 uL of 1% sulfuric acid. Then 10 uL of the Cobalt Sulfate reagent are dispensed into the 1% sulfuric acid and the two reagents are mixed by moving the plunger of the pipet dispenser (included in robotic arm 12) up and down several times.

This mode of dispensing ensures that the colorimetric reagent is completely dispensed. When attempts are made to dispense a 10-uL droplet of liquid onto a "dry" microtiter plate, the surface tension on the droplet

will cause the liquid to adhere to the tip of the pipet and consequently prevent a complete transfer of the reagent.

To ensure that volumes other than 10 uL are also accurately dispensed, the rest of the microtiter plate is filled with different volumes of the colorimetric reagent Cobalt Sulfate and its diluent 1% sulfuric acid as summarized below:

Row	uL H_2SO_4	uL $CoSO_4$	Comment
A1-A12	100 uL	0 uL	Blank by SUMMIT
B1-B12	90	10	dispensed by SUMMIT
C1-C12	80	20	dispensed by SUMMIT
D1-D12	70	30	dispensed by SUMMIT
E1-E12	60	40	dispensed by SUMMIT
F1-F12	50	50	dispensed by SUMMIT
G1-G12	0	100	dispensed by SUMMIT
H1-H12	0	100	by reference pipette

The software assumes that

i) The volume pipetted into wells H1-H12 is correct. These wells are pipetted manually using a calibrated pipet.

ii) The absorbance (A) of Cobalt Sulfate, in 1% sulfuric acid, is directly proportional to its concentration (Beer's Law).

Therefore, the volume of Cobalt Sulfate solution (V) delivered to each well is calculated as:

V = (Absorbance measured/Av Absorbance H1 - H12) x Volume of Calibrated Pipette

The software also allows for the correction of the error in the reference pipet. The calculated volume is compared with the gravimetrically calibrated volume instead of the nominal volume of the reference pipet as shown in the above equation. To obtain absorbance measurement of the cobalt sulfate reagent, the filled microtiter plate is read at 492 nM.

Software of this invention performs all the necessary data collection and calculations. A report is generated and a sample report is attached. The details of the software may be best understood from the flow chart of Figure 3.

SYSTEM SOFTWARE

The IBM Computer (IBM AT or IBM PS2/50) interfaces with the SUMMIT (TM) and an automatic microplate reader through two separate serial interface ports.

The software program sends command sequences to the SUMMIT controller electronics, effectuates the pipetting of samples and receives absorbance data from the microplate reader.

SYSTEM OPERATION

To begin the verification process, the user will access the software program and select the "Volumes Verification Menu" (Figure 4.a). Normally the user will then select the "Start Volumes Verification" option.

The software program is interactive and instructs the user to perform certain tasks during the verification process. For example, the software program will instruct the user to place the appropriate reagents at the proper locations by showing a depiction of the SUMMIT (TM) reagent rack (Figure 4.b). If the user wishes to stop the procedure, he may choose the "cancel" option at any time. The junctions for the "cancel" options are shown in Figures 4.b to 4.g.

After the reagents are placed in the correct locations, the user will continue the verification process by selecting the "Fill Verification Plate" option. A series of commands will be sent to the SUMMIT and strips A through G will be filled automatically.

When rows of wells A through G are completely filled, the SUMMIT instrument will stop and the software program will tell the user to fill strip H with a calibrated reference pipet. Once this step is completed, the user will place the microtiter plate into the microtiter reader by selecting the "Read Verification Plate" option. Data collection will be performed automatically and the data will be displayed on the screen. If an error occurs during

the data collection step, the user has the option to repeat the data collection process by selecting the "Reread Verification Plate" option (Figure 4.c). Various calculations will be performed and the user has the option to print a summary report or a full report with graphs. Data will be saved automatically on the system hard-disk and can be recalled anytime by selecting the "View Old Verification Plate" option (Figure 4.d).

For record keeping purposes, the user will store the serial number of the SUMMIT, the reagent lot number and the reagent expiration date in the system by using the "View/Edit Verification Reagent Data" option (Figure 4.e).

INSTRUMENT CONTROL AND CALCULATIONS

Details for the instrument control functions of the software program are shown in Figures 4.f and 4.g. During the "Initialize SUMMIT" step, commands will be sent to the SUMMIT to find the starting position for the stepper motors. Twelve disposable pipet tips will then be loaded and the plate will be filled automatically. The user may choose to abort the filling process at anytime by selecting the cancel option. Details of the filling procedure for individual strips are shown in Figure 4.g. During the process of filling each strip, the software program will instruct the SUMMIT to aspirate the reagent, wipe the tips and pipette the reagent into the microtiter plate. The amounts of cobalt sulfate and sulfuric acid required for each strip are shown in Figure 4.f. Details of the calculation algorithm and the report printing format are shown on Figures 4.h and 4.i, respectively.

The method described is a relatively simple yet highly accurate means for determining the accuracy of the automatic dispensing pipets. It does not rely upon the accuracy of a particular reagent but rather relies upon comparisons made to a reference.

Claims

1. A method of determining the accuracy of a liquid dispensing pipet using a plurality of wells of the same configuration and volume by the steps of:
   introducing a known volume of a colorimetric reagent of a known concentration that absorbs radiant energy in a manner directly proportional to its concentration into a first well,
   dispensing a first desired volume of the colorimetric reagent using the liquid dispensing pipet into a second well,
   measuring the absorbance of the reagent in each of the wells, and
   calculating the actual volume of reagent dispensed in the second well by multiplying the know volume by the ratio of the absorbance of reagent in the second well to the absorbance of the reagent in the first well.

2. The method of claim 1 which includes the additional step of comparing the calculated actual volume with the desired volume.

3. The method of claim 1 wherein any stable colorimetric reagent such as cobalt sulfate or potassium dichromate can be used.

4. The method of claim 1 which includes the additional steps of dispensing a second desired volume of the colorimetric reagent together with a first desired volume of a diluent for the reagent into a third well, and calculating the actual volume of reagent dispensed in the third well by multiplying the known volume by the ratio of the absorbance of the reagent in the third well to the absorbance of the reagent in the first well.

5. In the method of claim 4 wherein the diluent is sulfuric acid.

6. In the method of claim 2 which includes the additional steps of dispensing a second desired volume of the colorimetric reagent together with a first desired volume of a diluent for the reagent into a third well, and calculating the actual volume of reagent dispensed in the third well by multiplying the known volume by the ratio of the absorbance of the reagent in the third well to the absorbance of the reagent in the first well.

7. In the method of claim 6 wherein the colorimetric reagent is cobalt sulfate.

8. In the method of claim 3 wherein a plurality of first wells in a row are each dispensed with a known volume

of a colorimetric reagent of a known concentration, and a plurality of second wells forming additional rows of wells are each dispensed with the desired volume of different concentrations of the colorimetric reagent, calculating the actual volume of each of the different desired volumes dispensed by multiplying the known volume by the ratio of the absorbance of reagent in each dispensed well to the average absorbance of the first row of wells.

Patentansprüche

1. Verfahren zur Bestimmung der Genauigkeit einer Pipette zur Verteilung von Flüssigkeiten unter Verwendung mehrerer Vertiefungen derselben Bauform und desselben Volumens durch folgende Schritte:
   - Einfüllen eines bekannten Volumens eines colorimetrischen Reagenzes bekannter Konzentration, das Strahlungsenergie in einer Menge direkt proportional zu seiner Konzentration absorbiert, in eine erste Vertiefung;
   - Einfüllen eines ersten gewünschten Volumens des colorimetrischen Reagenzes mit Hilfe der Pipette zum Verteilen von Flüssigkeiten in eine zweite Vertiefung;
   - Messen des Absorptionsvermögens des Reagenzes in beiden Vertiefungen und
   - Berechnung des tatsächlichen Volumens des in die zweite Vertiefung eingefüllten Reagenzes durch Multiplizieren des bekannten Volumens mit dem Verhältnis des Absorptionsvermögens des Reagenzes in der zweiten Vertiefung zum Absorptionsvermögen des Reagenzes in der ersten Vertiefung.

2. Verfahren gemäß Anspruch 1, das den zusätzlichen Schritt des Vergleichens des berechneten tatsächlichen Volumens mit dem gewünschten Volumen umfaßt.

3. Verfahren gemäß Anspruch 1, wobei jedes stabile colorimetrische Reagenz, wie Cobaltsulfat oder Kaliumdichromat, verwendet werden kann.

4. Verfahren gemäß Anspruch 1, das die zusätzlichen Schritte des Einfüllens eines zweiten gewünschten Volumens des colorimetrischen Reagenzes zusammen mit einem ersten gewünschten Volumen eines Verdünnungsmittels für das Reagenz in eine dritte Vertiefung und der Berechnung des tatsächlichen Volumens des in die dritte Vertiefung eingefüllten Reagenzes durch Multiplizieren des bekannten Volumens mit dem Verhältnis des Absorptionsvermögens des Reagenzes in der dritten Vertiefung zum Absorptionsvermögen des Reagenzes in der ersten Vertiefung umfaßt.

5. Verfahren gemäß Anspruch 4, wobei das Verdünnungsmittel Schwefelsäure ist.

6. Verfahren gemäß Anspruch 2, das die zusätzlichen Schritte des Einfüllens eines zweiten gewünschten Volumens des colorimetrischen Reagenzes zusammen mit einem ersten gewünschten Volumen eines Verdünnungsmittels für das Reagenz in eine dritte Vertiefung und der Berechnung des tatsächlichen Volumens des in die dritte Vertiefung eingefüllten Reagenzes durch Multiplizieren des bekannten Volumens mit dem Verhältnis des Absorptionsvermögens des Reagenzes in der dritten Vertiefung zum Absorptionsvermögen des Reagenzes in der ersten Vertiefung umfaßt.

7. Verfahren gemäß Anspruch 6, wobei das colorimetrische Reagenz Cobaltsulfat ist.

8. Verfahren gemäß Anspruch 3, wobei mehrere erste Vertiefungen in einer Reihe jeweils mit einem bekannten Volumen eines colorimetrischen Reagenzes bekannter Konzentration befüllt werden und mehrere zweite Vertiefungen, die zusätzliche Reihen von Vertiefungen bilden, jeweils mit dem gewünschten Volumen verschiedener Konzentrationen des colorimetrischen Reagenzes befüllt werden, sowie Berechnung des tatsächlichen Volumens jedes der verschiedenen eingefüllten gewünschten Volumina durch Multiplizieren des bekannten Volumens mit dem Verhältnis des Absorptionsvermögens des Reagenzes in jeder befüllten Vertiefung zum Mittelwert des Absorptionsvermögens der ersten Reihe von Vertiefungen.

Revendications

1. Un procédé de détermination de la précision d'une pipette de distribution de liquide utilisant une pluralité

de puits de même configuration et même volume en suivant les étapes consistant à:

- introduire un volume connu d'un réactif colorimétrique d'une concentration connue qui absorbe l'énergie radiante de façon directement proportionnelle à sa concentration dans un premier puits;
- distribuer un premier volume souhaité du réactif colorimétrique en utilisant la pipette de distribution du liquide dans un second puits;
- mesurer le coefficient d'absorption du réactif dans chacun des puits; et
- calculer le volume effectif de réactif distribué dans le second puits en multipliant le volume connu par le rapport du coefficient d'absorption du réactif dans le second puits au coefficient d'absorption du réactif dans le premier puits.

2. Le procédé selon la revendication 1, qui comprend l'étape supplémentaire consistant à comparer le volume effectif avec le volume souhaité.

3. Le procédé selon la revendication 1, dans lequel on peut utiliser tout réactif colorimétrique stable tel que sulfate de cobalt ou dichromate de potassium.

4. Le procédé selon la revendication 1, qui comprend les étapes supplémentaires consistant à distribuer un second volume souhaité de réactif colorimétrique en même temps qu'un premier volume souhaité d'un diluant pour le réactif dans un troisième puits, et à calculer le volume effectif de réactif distribué dans le troisième puits en multipliant le volume connu par le rapport du coefficient d'absorption du réactif dans le troisième puits au coefficient d'absorption du réactif dans le premier puits.

5. Procédé selon la revendication 4, dans lequel le diluant est l'acide sulfurique.

6. Procédé selon la revendication 2, qui comprend les étapes supplémentaires consister à:
   - distribuer un second volume souhaité de réactif colorimétrique en même temps qu'un premier volume souhaité d'un diluant pour le réactif dans un troisième puits; et
   - calculer le volume effectif de réactif distribué dans le troisième puits en multipliant le volume connu par le rapport du coefficient d'absorption du réactif dans le troisième puits au coefficient d'absorption du réactif dans le premier puits.

7. Procédé selon la revendication 6, dans lequel le réactif colorimétrique est le sulfate de cobalt.

8. Procédé selon la revendication 3, dans lequel, à une pluralité de premiers puits dans une rangée, on distribue à chacun un volume connu d'un réactif colorimétrique d'une concentration connue et à une pluralité de seconds puits formant des rangées supplémentaires de puits, on distribue à chacun le volume souhaité de concentrations différentes de réactif colorimétrique, calculant le volume effectif de chacun des différents volumes souhaités distribués en multipliant le volume connu par le rapport du coefficient d'absorption du réactif dans chaque puits distribué au coefficient d'absorption moyen de la première rangée de puits.

Fig. 1

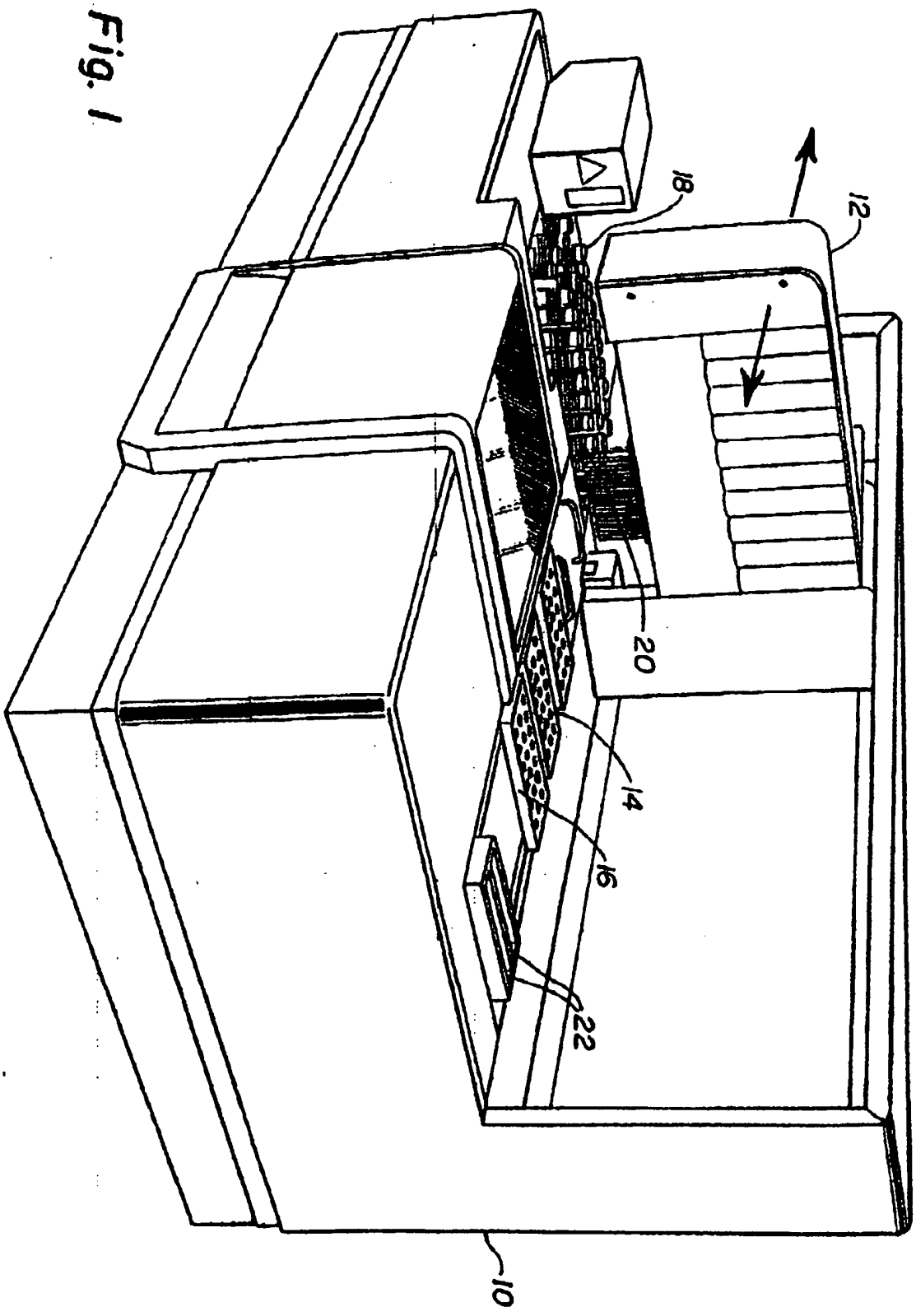
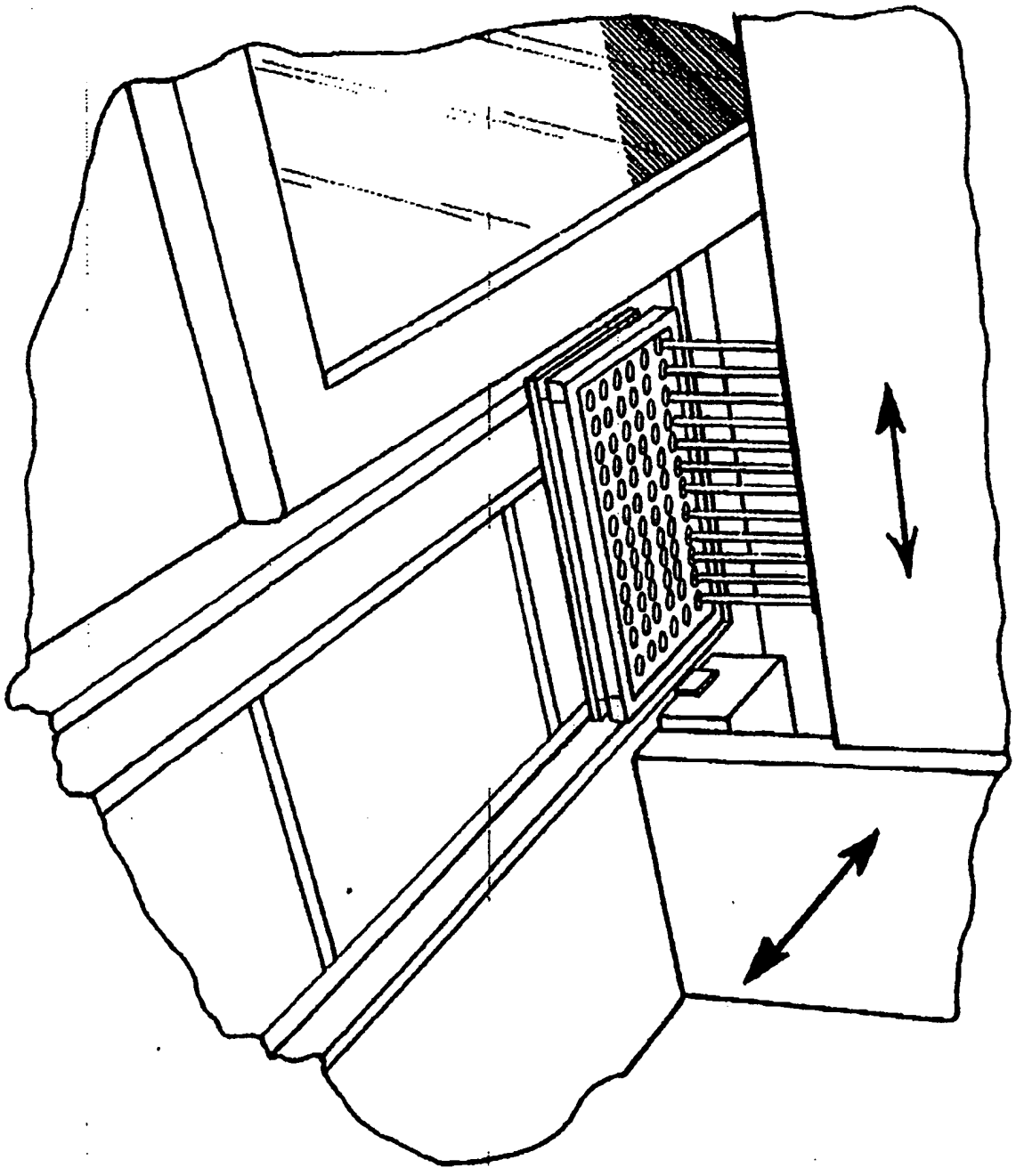


Fig. 2



VERIFICATION SYSTEM LAYOUT

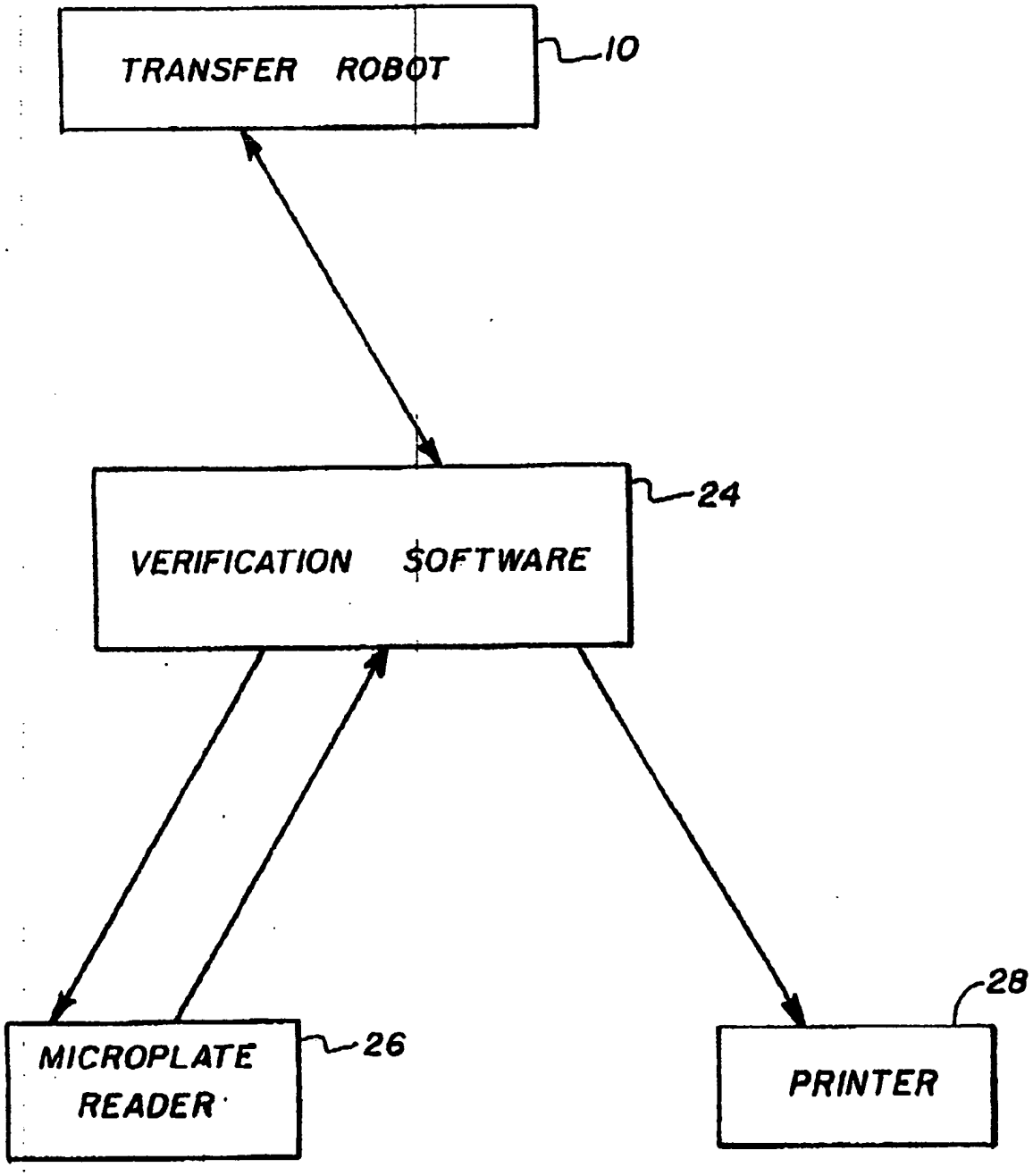


Fig. 3

Fig. 4a

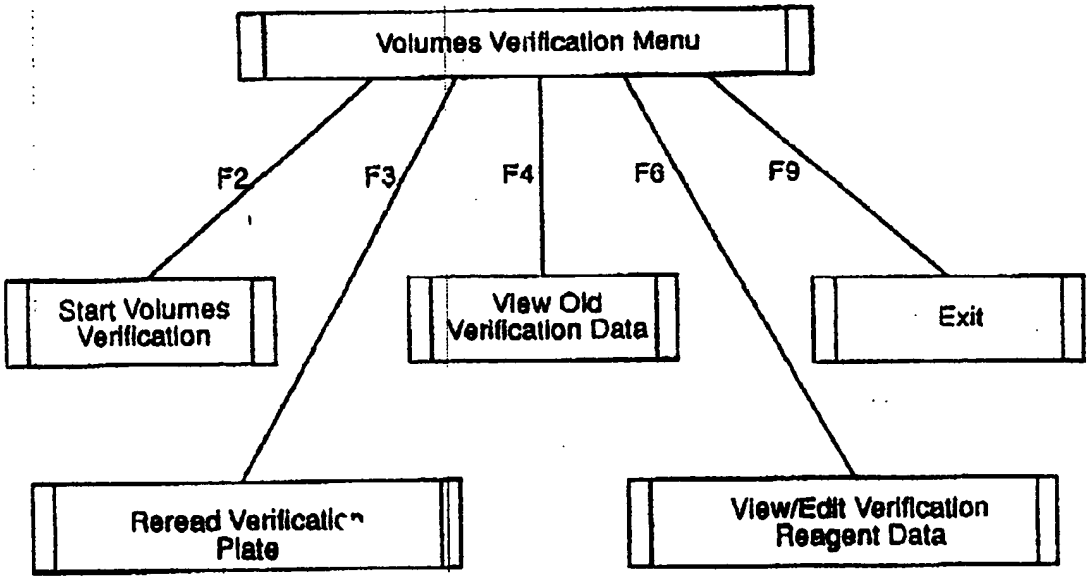
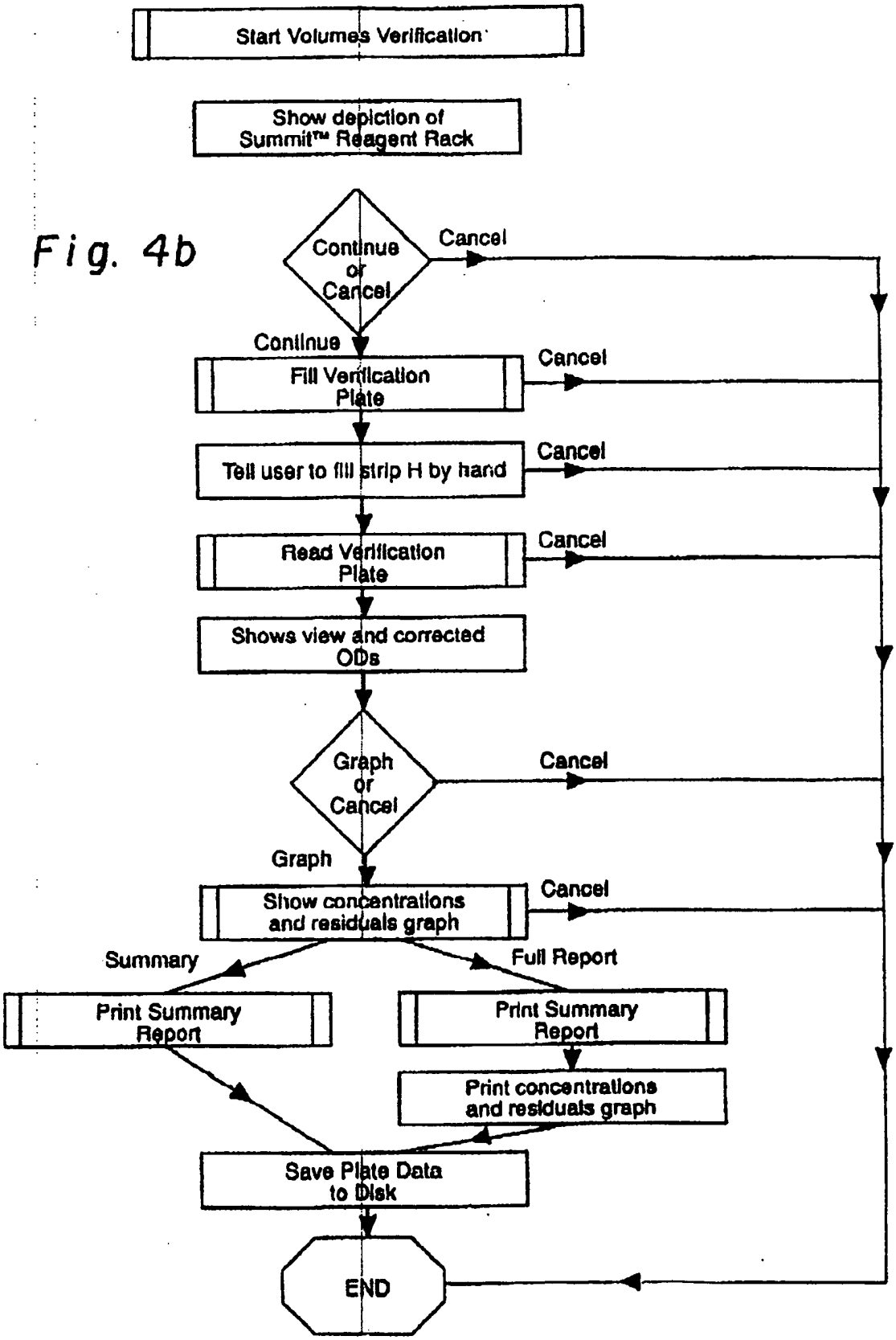
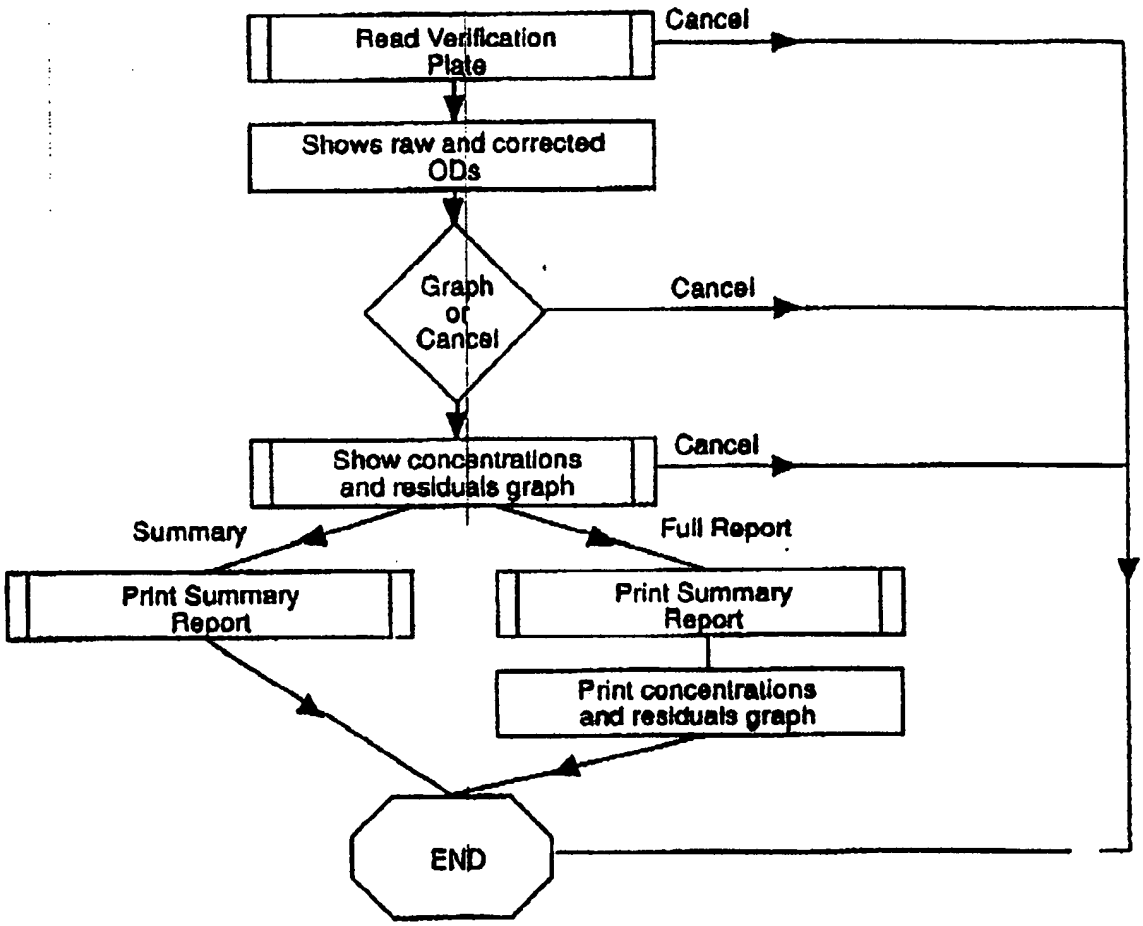

Volumes Verification Menu
F2
F3
F4
F6
F9
Start Volumes Verification
View Old Verification Data
Exit
Reread Verification Plate
View/Edit Verification Reagent Data

Fig. 4b


Start Volumes Verification
Show depiction of Summit™ Reagent Rack
Continue or Cancel
Cancel
Continue
Fill Verification Plate
Cancel
Tell user to fill strip H by hand
Cancel
Read Verification Plate
Cancel
Shows view and corrected ODs
Graph or Cancel
Cancel
Graph
Show concentrations and residuals graph
Cancel
Summary
Full Report
Print Summary Report
Print Summary Report
Print concentrations and residuals graph
Save Plate Data to Disk
END

Reread Verification Plate

Fig. 4c


Read Verification Plate
Cancel
Shows raw and corrected ODs
Graph or Cancel
Cancel
Show concentrations and residuals graph
Cancel
Summary
Full Report
Print Summary Report
Print Summary Report
Print concentrations and residuals graph
END

View Old Verification Plate

Fig. 4d

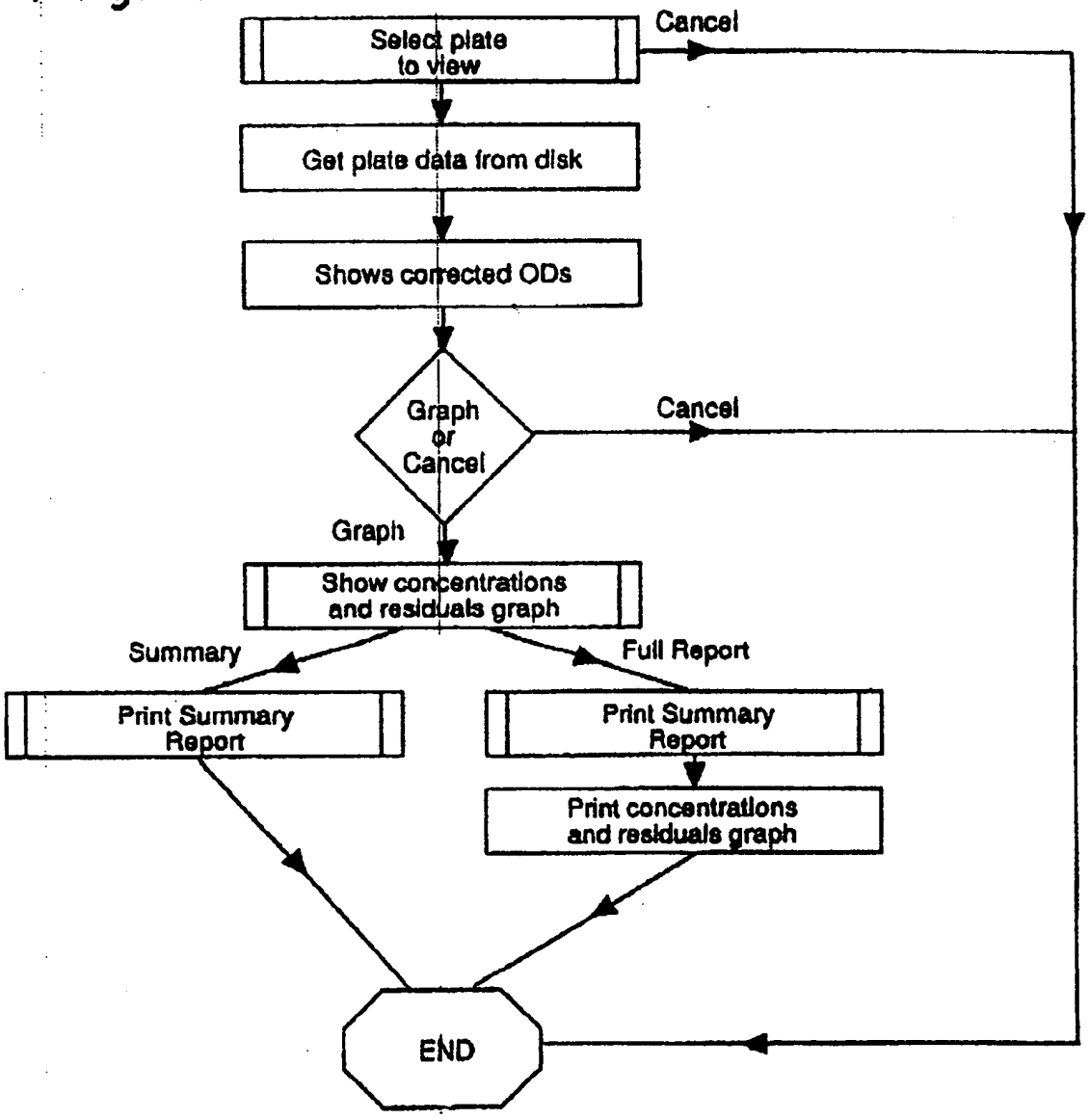
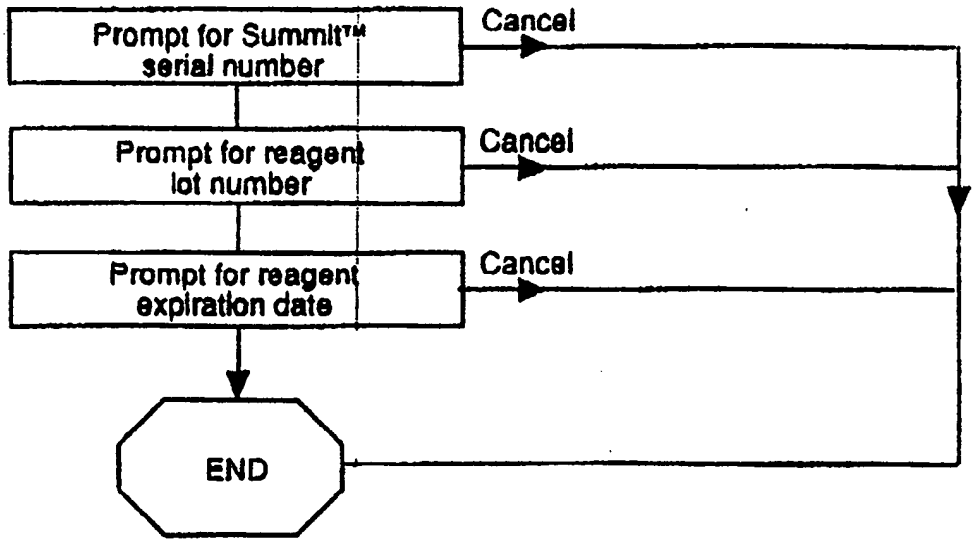

Select plate to view
Cancel
Get plate data from disk
Shows corrected ODs
Graph or Cancel
Cancel
Graph
Show concentrations and residuals graph
Summary
Full Report
Print Summary Report
Print Summary Report
Print concentrations and residuals graph
END

Fig. 4e

View/Edit Verification Reagent Data


Prompt for Summit™ serial number
Cancel
Prompt for reagent lot number
Cancel
Prompt for reagent expiration date
Cancel
END

Fill Verification Plate

Fig. 4f

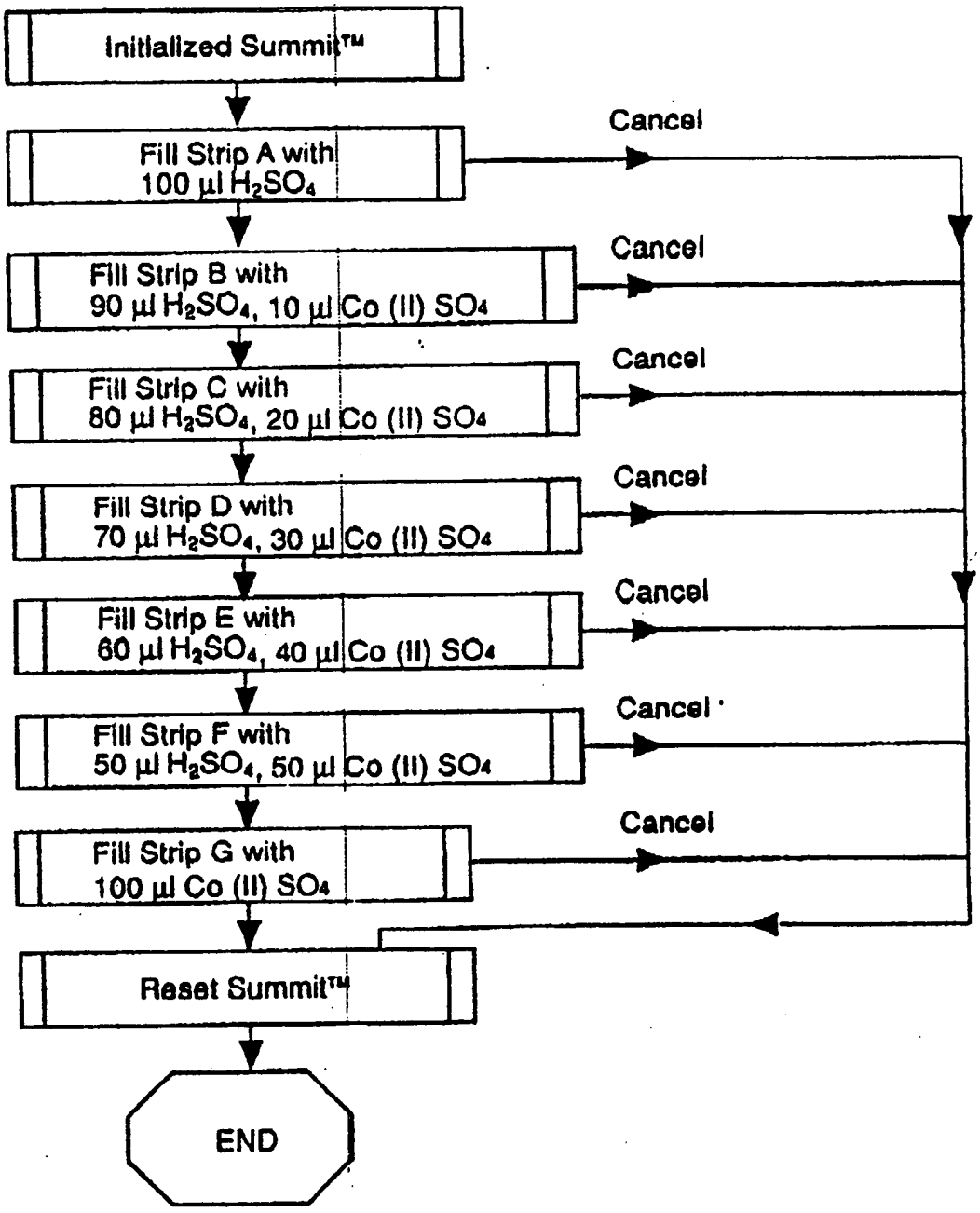

Initialized Summit™
Fill Strip A with 100 µl H_2SO_4
Cancel
Fill Strip B with 90 µl H_2SO_4, 10 µl Co (II) SO_4
Cancel
Fill Strip C with 80 µl H_2SO_4, 20 µl Co (II) SO_4
Cancel
Fill Strip D with 70 µl H_2SO_4, 30 µl Co (II) SO_4
Cancel
Fill Strip E with 60 µl H_2SO_4, 40 µl Co (II) SO_4
Cancel
Fill Strip F with 50 µl H_2SO_4, 50 µl Co (II) SO_4
Cancel
Fill Strip G with 100 µl Co (II) SO_4
Cancel
Reset Summit™
END

Fig. 4g

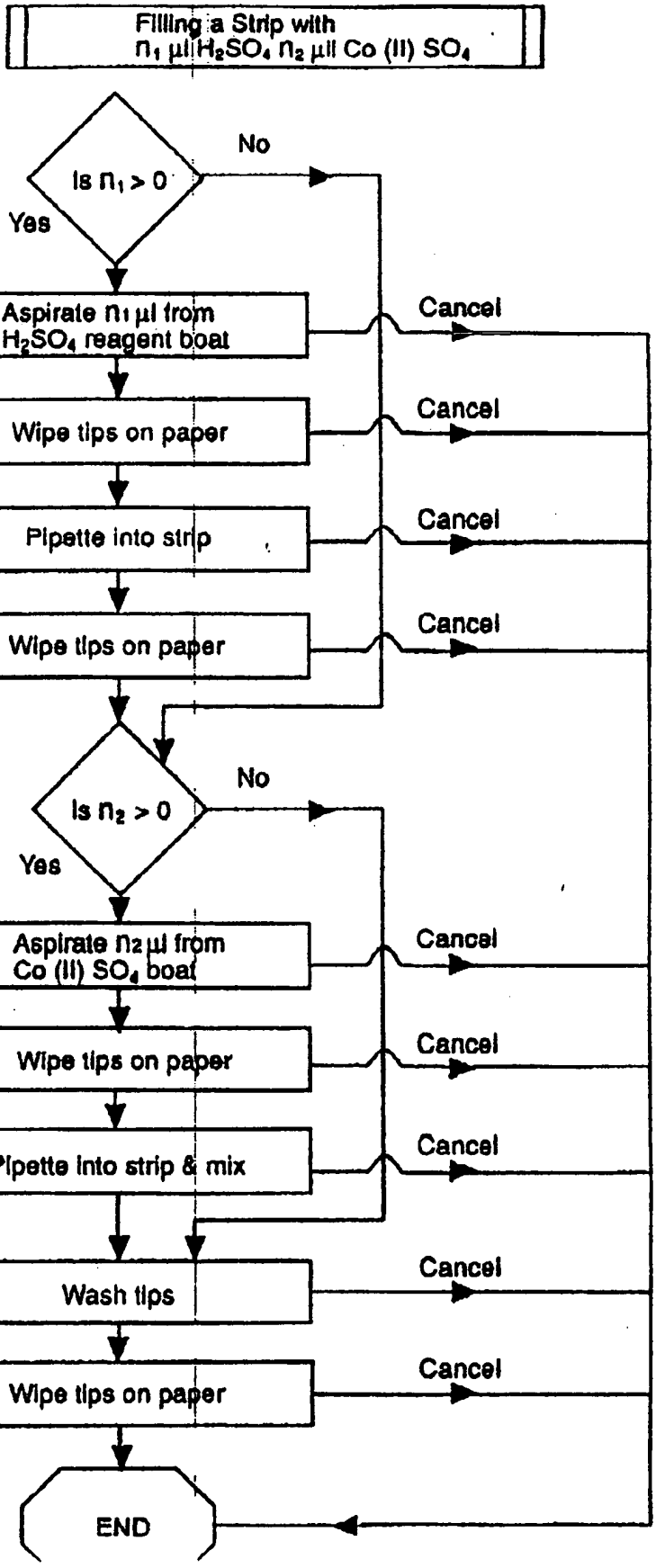

Filling a Strip with
n1 µl H2SO4 n2 µl Co (II) SO4
Is n1 > 0
No
Yes
Aspirate n1 µl from
H2SO4 reagent boat
Cancel
Wipe tips on paper
Cancel
Pipette into strip
Cancel
Wipe tips on paper
Cancel
Is n2 > 0
No
Yes
Aspirate n2 µl from
Co (II) SO4 boat
Cancel
Wipe tips on paper
Cancel
Pipette into strip & mix
Cancel
Wash tips
Cancel
Wipe tips on paper
Cancel
END

Fig. 4h

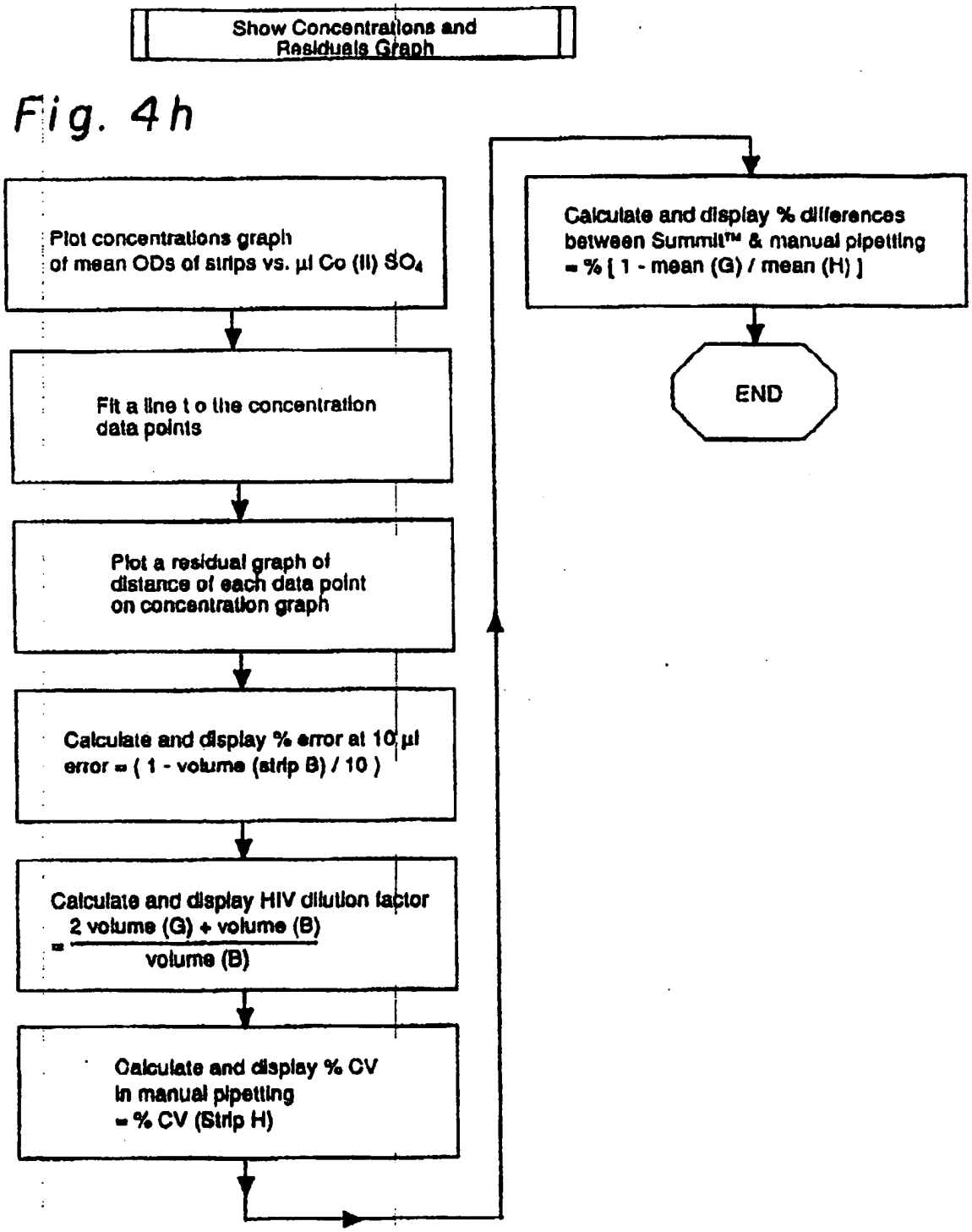
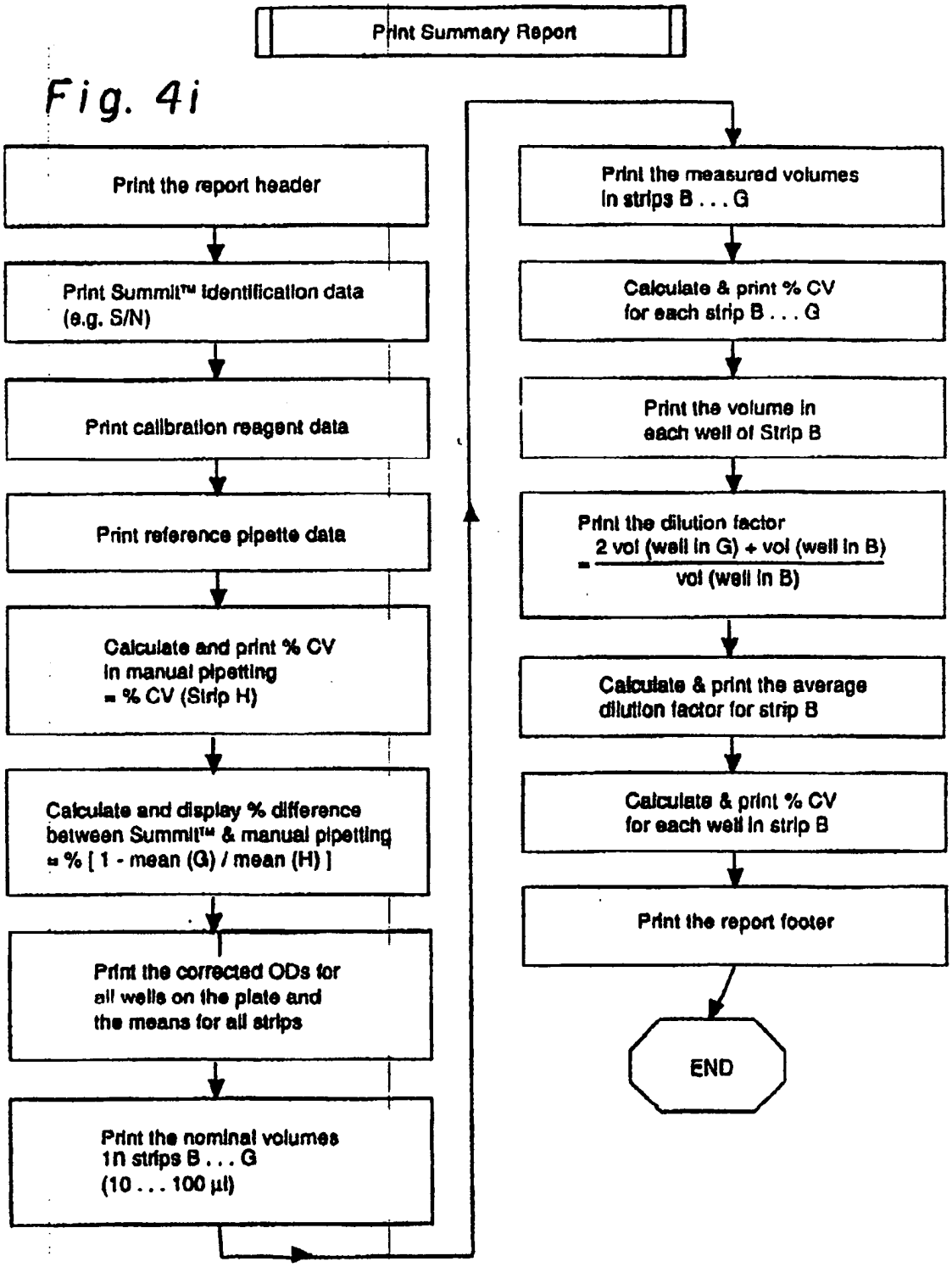

Show Concentrations and Residuals Graph
Plot concentrations graph of mean ODs of strips vs. µl Co (II) SO_4
Fit a line t o the concentration data points
Plot a residual graph of distance of each data point on concentration graph
Calculate and display % error at 10 µl error = (1 - volume (strip B) / 10)
Calculate and display HIV dilution factor = 2 volume (G) + volume (B) / volume (B)
Calculate and display % CV in manual pipetting = % CV (Strip H)
Calculate and display % differences between Summit™ & manual pipetting = % [1 - mean (G) / mean (H)]
END

Fig. 4i


Print Summary Report
Print the report header
Print Summit™ identification data (e.g. S/N)
Print calibration reagent data
Print reference pipette data
Calculate and print % CV in manual pipetting = % CV (Strip H)
Calculate and display % difference between Summit™ & manual pipetting = % [1 - mean (G) / mean (H)]
Print the corrected ODs for all wells on the plate and the means for all strips
Print the nominal volumes in strips B . . . G (10 . . . 100 µl)
Print the measured volumes in strips B . . . G
Calculate & print % CV for each strip B . . . G
Print the volume in each well of Strip B
Print the dilution factor = 2 vol (well in G) + vol (well in B) / vol (well in B)
Calculate & print the average dilution factor for strip B
Calculate & print % CV for each well in strip B
Print the report footer
END

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(71) Applicant: **E.I. DU PONT DE NEMOURS AND COMPANY**
1007 Market Street
Wilmington Delaware 19898(US)

(72) Inventor: **Lung, Kaki Rick**
48 Quail Hollow Drive
Hockessin, Delaware 19707(US)
Inventor: **Fok, John Shiu-Kang**
41 Slashpine Circle
Hockessin, Delaware 19707(US)
Inventor: **Chanin, Alex Joseph**
8837 Rising Sun Avenue
Philadelphia, Pennsylvania 19115(US)

(74) Representative: **von Kreisler, Alek,**
Dipl.-Chem. et al
Patentanwälte Von Kreisler-Selting-Werner,
Deichmannhaus am Hauptbahnhof
W-5000 Köln 1(DE)

(54) **Method for verifying the accuracy of a liquid dispenser.**

(57) This invention relates to a method of determining the accuracy of a liquid dispenser. More particularly, this invention relates to a method of use of robotic equipment in the processing of microliter-plate based assays. The method permits the diagnostic instruments using absorbance type measurements and laboratory robots in the analysis of samples to verify the accuracy of the dispensing portion of the robot by comparing the absorbance due to a dispensed volume of reagent with that due to a known volume of the same reagent. This method finds particular use in the diagnostic instruments analyzing samples which contain viruses (or antibody to the virus) of infectious diseases. Typical of such infectious diseases are the human immunodeficiency virus Type -1(HIV-1).

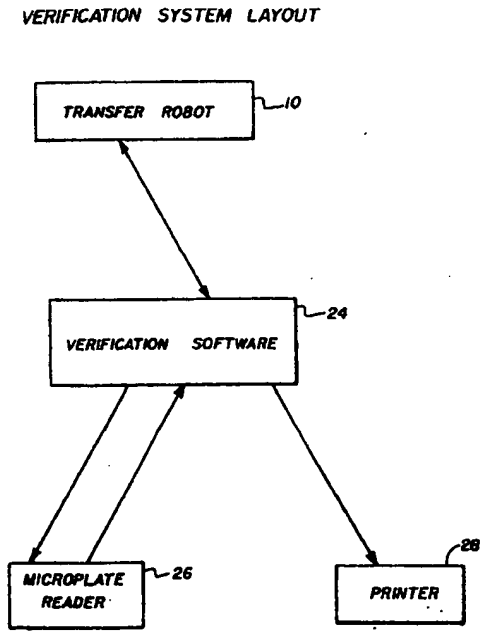

Fig. 3

EP 0 431 578 A3

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 12 3279

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
D,Y	US-A-4 354 376 (W. GREENFIELD ET AL.) * column 4, line 58 - column 5, line 49 *	1,2,4,6	G01N35/06 G01N21/25
P,Y	EP-A-0 374 686 (BEHRINGWERKE) 27 June 1990 * page 3, line 25 - page 5, line 25 *	1,2,4,6	
A	US-A-4 586 546 (L.M. MEZEI ET AL.) * column 8, line 31 - column 9, line 36 *	1,2,4,6	
A	US-A-4 772 487 (Y. GOTOH ET AL.) * column 4, line 53 - column 5, line 20 *	1	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

G01N
G01F
B01L

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	14 NOVEMBER 1991	HODSON C.M.

CATEGORY OF CITED DOCUMENTS

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EPO FORM 1503 03.82 (P0401)